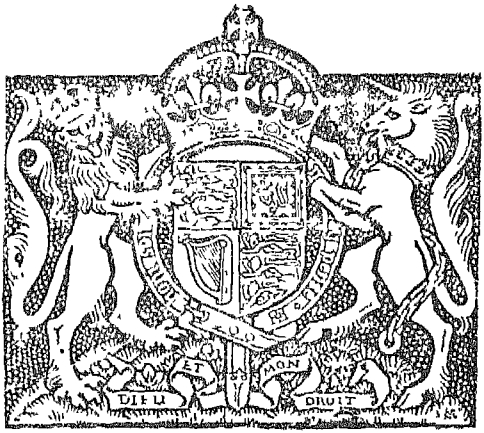

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THE MICROSCOPE

THE MICROSCOPE

AND THE PRACTICAL PRINCIPLES OF OBSERVATION

by

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FOREWORD

Since its invention, the microscope has made itself indispensable in an ever growing number of fields of research until, at the present time, there is scarcely a science or an industry that does not depend on it to a greater or lesser degree for its advancement.

Working with the microscope is not however as simple as is often thought, and to get the most out of this instrument a careful technique must be followed. Should this be neglected, there is the risk not only of missing many essential details, but—graver still—of being misled by false appearances and optical illusions.

Most books on microscopy, which describe the preparation and staining of animal and plant tissues and other objects for microscopical study, give little or no instruction as to their examination when actually upon the microscope stage.

The present volume attempts to meet this want, and presents a methodical survey of the technique of microscopical *observation*. It was primarily written from the medical angle, but the fundamental principles remain the same for all the other branches of microscopy as a whole.

The author lays no claim to new methods, but, by collecting the procedures and techniques found in a great number of different works, by unifying them and incorporating certain details suggested by his own experience, he hopes to have compiled a handbook that will smooth the path of the beginner and perhaps also be of some value to the more advanced worker with the microscope.

Grateful appreciation is tendered to Dr. and Mrs. C. N. Partington for their valuable suggestions in connection with this book, and thanks are due to the Optical Firms, Messrs. W. Watson and Messrs R. & J. Beck, who kindly supplied the blocks for Figs. 1, 2 and 18.

PART ONE

DESCRIPTION OF THE MICROSCOPE

CHAPTER I

THE STAND

The microscope comprises two essential parts: The *Stand* and the *Optical System.*

The stand carries the optical system. It should be of firm and rigid construction so as not to be subject to distortions, to play in its component parts and to vibrations which would be disastrous to all useful work. It consists of the following parts: (1) The Foot. (2) The Limb. (3) The Body. (4) The Focusing Mechanism. (5) The Stage. (6) The Substage. (7) The Mirror.

1. THE FOOT

Two forms of foot are in common use: The *tripod,* sometimes called the 'English model', and the *horseshoe* or 'Continental model'.

The tripod is lighter, less subject to vibration and more stable, especially when the microscope is inclined to the horizontal for drawing or photomicrography.

The horseshoe is more compact and allows the microscope to be packed in a smaller case. The microscope can also be brought nearer to the edge of the table without the danger of one of the feet being pushed over the side. It is consequently a more practical model for the busy worker.

The foot consists of two parts: the *base* and the *pillar.* In most modern microscopes these are cast in one piece for greater rigidity. The foot should be properly proportioned for the microscope to be well balanced in the inclined positions which are most convenient for working. To obtain this result in the horseshoe model, the spur at the rear of the base should be fairly large and the latter should rest on the table by only three points of contact.

2. THE LIMB

The limb carries the microscope body; it is hollow and contains the focusing mechanism. As with the foot, it should be of solid construction to avoid stresses and vibration. Its shape should allow it to be used as a lifting handle without straining any of the mechanical parts. This is attained in all modern microscopes, but some of the older models, still frequently met with, should not be lifted by the limb but by the foot and pillar. If this is not done, the fine adjustment may be strained and, on putting down the microscope, the finger may be pinched in the slot around the lower end of the limb.

The limb is joined to the pillar by the axis joint, except in some small non-inclinable dissecting microscopes. It should be possible to adjust or lock this joint by means of a built-in clamping lever; otherwise an adjusting spanner should be provided with the instrument.

3. THE BODY

The body is composed of two parts: an external *body-tube* and an internal *drawtube.*

The *body-tube* is provided at its upper end with a smooth ring in which the drawtube slides. Its lower end is fitted with a female thread to take objectives of the Royal Microscopical Society's standard gauge (see page 25).

The modern tendency is to give the body-tube a large diameter, 5 cm. (2 in.) or more, to avoid as much as possible all internal reflections, especially when the microscope is used for photomicrography. This is not indispensable however as a diaphragm suitably placed in the drawtube (see page 55) can cut off any stray light.

The *drawtube* is one of the most important parts of the microscope. Its function is not to vary the magnification, as is often thought, but to neutralize the spherical aberration introduced by cover-glasses of a different thickness from that for which an objective has been corrected.

It should slide very smoothly in its sleeve which should be cloth-lined. This model is by far the best as all metal sleeves tarnish after a time and a smooth movement cannot be obtained.

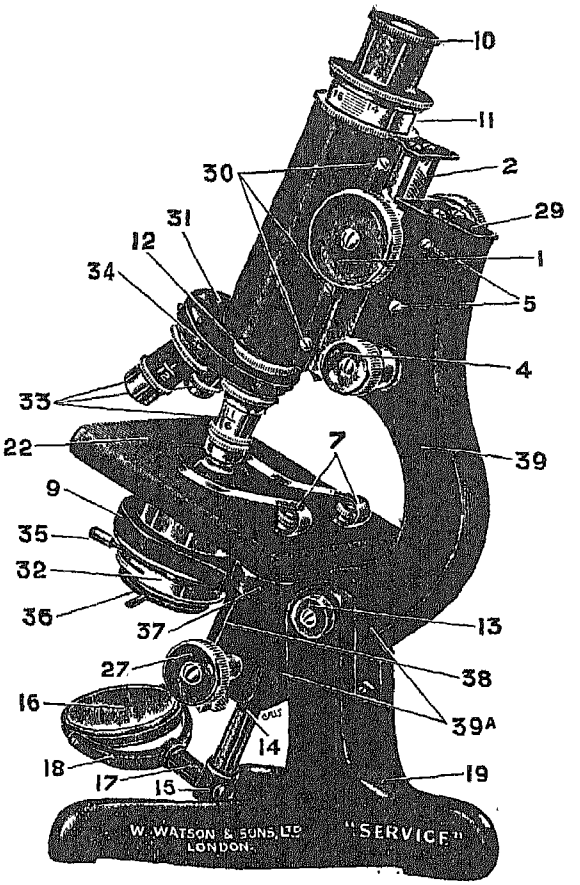

FIG. 1. Parts of a Microscope

(1) Fine adjustment milled heads. (2) Coarse adjustment rackwork. (4) Fine adjustment milled heads. (5) Fine adjustment compensating screws. (7) Stage clips. (9) Substage ring. (10) Eye piece. (11) Drawtube. (12) R.M.S. female objective thread. (13) Axis joint. (14) Mirror tail-piece. (15) Mirror fitting. (16) Mirror. (17) Mirror arm. (18) Mirror gymbal (19) Foot. (22) Stage. (27) Substage coarse adjustment milled head. (29) Plate covering fine adjustment spring box. (30) Coarse adjustment compensating screws. (31) Revolving triple nosepiece. (32) Condenser. (33) Objectives. (34) Tangential centring screws on nosepiece. (35) Iris diaphragm lever. (36) Condenser stop carrier. (37) Bracket carrying stage. (38) Substage. (39—39A) Optical bench pattern limb.

(*Note.* The numbers 3, 6, 8, 20, 21, 23, 24, 25, 26 and 28 are not included in the Figure.)

Should the coarse adjustment be at all loose, there is even the danger, when pushing in a stiff drawtube, of displacing the body and damaging the objective front-lens against the cover-glass. For the same reason, the drawtube should be enamelled or chromium-plated as ordinary brass tarnishes too rapidly. The drawtube should not however be loose enough to sink by its own weight or that of any accessory fixed to the eyepiece end.

It is very convenient if the drawtube is provided with a scale reading in millimetres to indicate the mechanical tube-length, i.e. the distance between the upper end of the drawtube and the lower end of the body where the objective is screwed.

The upper end of the drawtube receives the eyepiece. Its internal diameter should conform to the Royal Microscopical Society's gauge of 23·3 mm. (·9173 in.) which has been adopted by nearly all makers.

A well-made drawtube should be slightly larger in internal diameter at about 2·5 cm. (1 in.) from its upper end. If this is not the case, the inside of the drawtube is gradually polished by the low-power eyepieces which are longer than the high-power ones. This gives rise to troublesome internal reflections when the shorter oculars are used.

The lower end of the drawtube carries a diaphragm which should have a free diameter of at least 1·75 cm. (11/16 in.) so as not to cut off any of the light rays coming from the objective and thus reduce the numerical aperture of the latter. It is very convenient if this diaphragm is provided with a female thread into which objectives can be screwed, as in some microscopes the coarse adjustment rack has not sufficient range to enable objectives of 40 or 50 mm. (1 1/2—2 in.) to be focused. This difficulty is overcome if the objectives in question can be screwed into the lower end of the drawtube.

The drawtube should be able to give a total length (including that of the body and the rotating nosepiece) of about 200 mm. (7 7/8 in.) when it is pulled out and 140 mm. (5 1/2 in.) when it is pushed home. The above-mentioned lengths are the most convenient as, with thick cover-glasses, it is often necessary to shorten the mechanical tube-length to 140 or 150 mm. to get the best images. On the other hand, with very thin cover-glasses, a

length of 190 mm. or more may be required. It is also sometimes desirable not to use a cover-glass at all, and for this a very long tube-length is indispensable.

The inside of the drawtube and of the microscope body should be carefully blackened to reduce all internal reflections to a minimum. These can be still further prevented by placing a black cardboard diaphragm in the upper end of the drawtube, its position being determined by the length of the lowest power eye-piece used. (See page 55.)

Some of the larger microscope stands are fitted with a

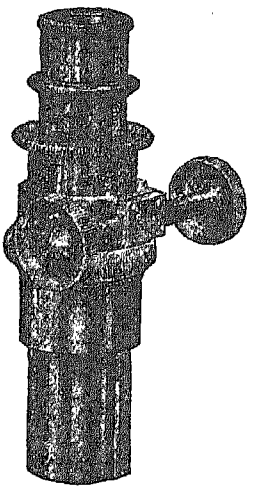

FIG. 2. Mechanical drawtube

mechanical drawtube consisting of two tubes sliding in each other. The outer tube is provided with a diagonal rack and pinion which can impart a very smooth motion to it. This accessory is very useful for obtaining the optimum tube-length with cover-glasses of different thicknesses, a manipulation of capital importance in some branches of research work, especially with high-power dark-ground condensers.

4. THE FOCUSING MECHANISM

The microscope is provided with two focusing mechanisms: the *coarse* and the *fine* adjustments.

The *coarse adjustment* mechanism consists of a diagonal rack and pinion, the latter being rotated by two large milled heads situated on either side of the upper end of the limb. These parts should work smoothly without any shake or back-lash. A good coarse adjustment ought to be delicate enough to focus a 4 mm. (1/6 in.) objective.

It is a great convenience in certain branches of work if the rack has a sufficiently long travel to enable objectives of as low a power as 40 mm. (1 1/2 in.) focal length to be focused when on the rotating nosepiece.

Compensating screws will be found very useful to obtain exactly the right degree of pressure between the rack and the pinion and to take up any looseness between the dovetail bearing and the female slide.

The *fine adjustment.* There are a great many different models of fine adjustment on the market. Most modern types are satisfactory provided that they are well made, but perhaps the most robust pattern is that which combines a micrometer screw with some form of horizontal or vertical lever. The vertical form is the most used nowadays as it has the advantage of having the fine adjustment milled heads on either side of the limb on an axis parallel with those of the coarse adjustment and slightly below the latter. This disposition allows the fingers to pass very conveniently from the one mechanism to the other during work. The horizontal lever is perhaps more satisfactory however in the long run as it only presents a plain up and down movement without any lateral complication. It is still used in some of the most modern microscopes and is actuated by a single milled head situated at the rear of the limb.

If the axes of the milled heads of both adjustments are parallel, they should both displace the body in the same direction when rotated the same way. If this is not the case much confusion and loss of time may be caused. Some patterns of fine adjustment incorporate an eccentric actuated by an endless screw. When these reach the lowest point of their course, further rotation of the milled heads in the *same* direction as before will cause the body to move *upwards* instead of downwards. To avoid all mishaps, the fine adjustment should lower the body-tube when

the milled heads are rotated clockwise, as is the case with the coarse adjustment, and the pointer engraved on the fine adjustment slide should always be kept more or less half-way between the two marks on the limb. This should also be done with the lever form of fine adjustment as with this the mechanism ceases to function when arriving at the end of its range.

The rim of one of the milled heads of the fine adjustment generally bears divisions to measure the vertical displacement of the microscope body. With most makers each division corresponds to a vertical displacement of 1/500 mm., thus permitting the *thickness* of the object examined to be ascertained. (See page 137.)

5. THE STAGE

The stage supports the object to be studied under the microscope. There are two patterns in common use: the *fixed square stage* and the *circular rotating stage.*

Whatever its shape, the stage should have a width of at least 9 to 10 cm. (3 1/2—4 in.) and its upper surface should be covered with ebonite or some similar incorrodible substance. The central aperture should have a diameter of 2·5 cm. (1 in.) or even more as otherwise, when using immersion condensers, the cedar oil is carried on to the upper surface of the stage when the object-slip is displaced during examination. The distance between the centre of the stage aperture and the limb should be at least 7·5 cm. (3 in.) to allow sections of large organs to be studied and Petri dishes, tanks and other apparatus to be used. The stage is provided with two spring-clips to hold the slide.

When the two patterns are compared, the fixed square stage has the advantage of being simpler and cheaper. It is also easier to fit with a detachable mechanical stage.

The circular rotating stage is, on the other hand, more convenient for drawing and photomicrography as it allows the object to be more easily orientated. It is also invaluable for metallurgy and all work with polarized light.

For medical and biological work the rotating stage has no particular advantage over the fixed square form and it is generally more difficult to fit with a detachable mechanical stage.

The fixed square stage is usually quite sufficient for all ordinary routine work.

The *fixed square stage* should comply with all the points enumerated above. It should also be thick enough not to give under the weight of the hand manipulating the slide otherwise the object will not remain in focus when high powers are used. Finally its construction should be such as to allow it to be easily fitted with a detachable mechanical stage.

The *circular rotating stage* should possess all the qualities mentioned previously. The best models are provided with two centring screws and a clamp. The function of the first two is to make the axis of rotation of the stage coincide with the optical axis of the objective used, that of the last to lock the rotation when desired.

Unfortunately most optical firms do not make detachable mechanical stages that can be fitted to the rotating stage. This is a point to be remembered when a choice has to be made.

When a rotating stage is provided with centring screws, it can be used as a mechanical stage to some extent, but its range of movement is too limited to explore the whole surface of a slide.

The Mechanical Stage

The mechanical stage is more than an accessory, it is in fact indispensable to all serious work as it allows a thorough and systematic exploration of a slide to be carried out. Two patterns are met with: the *detachable mechanical stage* and the *built-in mechanical stage.*

The *detachable mechanical stage* is designed to be adapted to a fixed square stage. There are many different models and methods of attachment. Some are fixed to the edge of the stage, others are fitted to the limb or else use is made of the holes for the spring-clips. The first are generally to be preferred as they are readily adapted to most microscope stands and can be more easily slipped into place or removed.

Whatever its pattern however, the mechanical stage should be sturdily built and free of all shake or vibration; its movements should be smooth without being too loose. The vertical movement should show no tendency to slide backwards from its own

weight when the microscope is inclined. The horizontal movement should have a range of at least 4 to 5 cm. (1 1/2—2 in.) and the vertical one of 2·5 cm. (1 in.). The distance between the two slide holders should be adjustable so as to take slides, compressors and other accessories of different sizes and shapes. One of the slide holders, preferably the left, should be provided with a tilting spring-clip with a sufficient arc for the fingers of the right hand to slide the object-slip easily into place even when objectives with a small working distance are being employed. The object examined must be firmly held or it will not follow the mechanical stage smoothly and continuously. This is particularly noticeable with oil-immersion objectives as, owing to the cedar oil, the cover-glass has a tendency to stick to the front-lens.

The *built-in mechanical stage*. The larger microscope stands are generally provided with a built-in mechanical stage. This pattern is by far the best as it presents more precision and stability in its movements than any detachable mechanical stage.

Two models are generally encountered: in the first the whole top of the stage is displaced for the vertical movement, but for the horizontal movement the object-slip alone is displaced and it is pushed along the surface of the stage exactly as in detachable mechanical stages. In the second model the stage is displaced all in one piece for both movements. This last pattern has many advantages over the other and deserves to be more widely adopted. It allows compressors, Petri dishes, tanks and slides of all shapes and sizes to be used with an ease and rapidity unattainable by other means.

The milled heads controlling the mechanical stage are placed laterally in some models, above the stage in others. The first type is the best as the fingers can pass more easily from the coarse and fine focusing milled heads to those of the mechanical stage if all their axes are parallel.

It is very convenient if the horizontal movement can be controlled at will by either hand by means of two milled heads placed on opposite sides of the stage. In this way both hands can be used simultaneously, one working the vertical and the other the horizontal movement, and the displacements of a

living organism swimming in a liquid medium can be much more easily followed.

The range of the mechanical stage should be at least 5 cm. (2 in.) for the horizontal and 2·5 cm. (1 in.) for the vertical movement.

Millimetre Scales and Verniers

There is some advantage in having the mechanical stage equipped with millimetric graduations and verniers as an interesting detail of an object-slip can then be recorded. Unfortunately this method is not always satisfactory in practice as, if there is the slightest play in the working parts, the desired detail will not be found again even when the scales have been set at the same numbers. It is not possible also to find an object logged by another microscope. There are other methods for recording the position of any detail of a slide; some of these are described subsequently on page 150.

6. THE SUBSTAGE

The substage comprises all the parts which carry, centre and focus the substage condenser or other illuminating apparatus. In its most elementary form it consists of a simple cylindrical tube attached to the under side of the stage. The condenser is moved up or down in this tube for focusing.

For more advanced work it is necessary to focus the condenser with greater accuracy and to centre it exactly with the optical axis of the objective employed; a more complex substage is consequently required.

The Mechanism for Focusing the Condenser

Two patterns of focusing mechanism are commonly met with. The first and cheapest consists of a large spiral screw by means of which the condenser can be raised or lowered. When the condenser arrives at the lowest point of its course, it is automatically swung aside to allow the mirror to be used alone when working with very low-power objectives. This mechanism is fairly satisfactory when well made, but it cannot give as good results as the second pattern in which the condenser is focused

by means of diagonal rack and pinion. The substage can also be provided with a fine adjustment for very delicate work.

In some stands the focusing mechanism for the condenser is attached to the under side of the stage, but in more modern microscopes it is carried on the lower end of the limb which is prolonged beneath the stage like an optical bench. This arrangement has everything to recommend it as, with the older form, the slight strain thrown upon the stage when focusing the condenser was sometimes enough to disturb the focus of a high power objective.

The Mechanism for Centring the Condenser

In all substages the condenser is carried by a cylindrical tube or ring into which it slides either from above or from below. The first form is the best as it obviates any chance displacement of the condenser by its own weight.

Small microscopes are not generally provided with any centring mechanism for the substage, but in larger stands the cylinder which carries the condenser is borne in an outer sleeve fitted with two screws working at right angles to each other against an antagonistic spring. With the aid of these *centring screws* the condenser can be accurately centred with the optical axis of the objective in use at the moment. This centring must be repeated whenever the objective is changed as the optical axes of all objectives differ from each other even when manufactured by the same firm.

The Calibre of the Substage Tube

The inside diameter of the cylinder which carries the condenser has been fixed by the Royal Microscopical Society at 38·786 mm. (1·527 in.). This gauge has been adopted by all British manufacturers, by the Spencer Lens Co. of America, and by Reichert of Vienna. Other optical firms have each a different gauge, and it is to be hoped that the R.M.S. diameter will soon become universal as has been the case with microscope objectives.

7. THE MIRROR

The mirror should have a diameter of at least 5 cm. (2 in.) and its centre should coincide exactly with the optical axis of the microscope. If these two conditions are not fulfilled, illumination may be unsatisfactory when the microscope is used in very inclined positions (see page 60).

Instead of being attached to the lower end of the substage rack, as is sometimes seen, it is preferable for the mirror to be carried on an independent rod or tail-piece. It should be possible to tilt the mirror at any angle and to move it up or down on its tail-piece so as to concentrate the maximum amount of light on to the object when the concave mirror is used by itself for low power work.

The mirror has two faces, plane and concave. The plane mirror is by far the more important of the two as it should *always* be used in conjunction with a condenser or any other form of illuminating apparatus. It should be optically worked to obtain two rigorously parallel surfaces otherwise multiple reflections of the light source may result with consequent deterioration of the microscope image. Some opticians supply *stainless steel* plane mirrors which completely eliminate multiple reflections; they are well worth their extra cost.

The concave mirror is used, without the condenser, for low powers. Its radius of curvature should be about 6 or 7 cm. (2 3/8—2 6/8 in.) so that an image of the light source may be focused in the plane of the object under examination and thus supply it with the maximum amount of light. Many of the microscope mirrors seen by the author had such a long focus that they could not be brought far enough away from the under side of the stage to give the best results. Tests for the mirror will be found in Chapter III.

OBJECTIVE CHANGERS

Some form of device for changing rapidly from one objective to another during work is such an indispensable adjunct to the microscope that it should be regarded as an *integral part of the body-tube* rather than as an accessory. A short description of

the two principal patterns of objective changer is accordingly given a place in this chapter.

The *rotating nosepiece* is the commonest form of objective changer and one which fulfils most requirements. It is made in three models, double, triple and quadruple, to bring respectively two, three or four objectives in line with the optical axis of the microscope. The choice will generally rest between a triple or a quadruple nosepiece, the other being only used on portable microscopes or on very small stands. The quadruple nosepiece has the advantage of allowing four objectives to be interchanged with greater ease, but it is somewhat more expensive and the weight of four objectives throws more strain on the focusing mechanism, especially the fine adjustment. This causes greater wear and tear in the long run.

All modern rotating nosepieces have a round upper plate to exclude the dust, the sole exception being the double model in which the dustproof upper plate is sometimes replaced by a simple cover for the objective which is not in use. This has the advantage of compactness, but the objectives are liable to gather dust if left by mistake only partially rotated.

The principal disadvantage of the rotating nosepiece is that it is exceptional for the optical axes of different objectives to coincide and consequently an object centred by one objective may not even appear in the field when another is rotated into position. This fault can be corrected to some extent in nosepieces which are provided with tangential adjusting screws. But even with these accuracy tends to deteriorate with use and the centring has, of course, to be carried out anew if the relative positions of the objectives are changed or if any of them are replaced by others.

The *sliding objective changer* overcomes the principal drawback of the rotating nosepiece. It consists of a tube fitting which is attached permanently to the lower end of the body-tube and an objective fitting which has also to be fixed permanently to the screw end of each objective used. The objective fitting slides into the tube fitting and is provided with adjusting screws by means of which all objectives can be accurately centred to the same optical axis.

With this pattern objectives can be quickly changed and the same object will always occupy the centre of the field. A substage condenser, when centred with the optical axis of one objective, will still be centred when that objective is replaced by another. Much time is thus saved, but it is advisable, even with the sliding objective changer, to check the condenser centring fairly frequently if the most accurate results are to be obtained.

The disadvantages of the sliding objective changer are: (a) the necessity of providing a special case to contain the objectives in their fittings to protect them from dust and damage; and (b) the higher cost and greater encumbrance of these various separate parts.

CHAPTER II

THE OPTICAL SYSTEM

The optical system of the microscope includes: (1) The Objective or Object-glass. (2) The Eyepiece or Ocular. (3) The Substage Condenser.

1. THE OBJECTIVE

The objective is composed of the *mount* and the *lenses.*

The *mount* consists of a metal cylinder carrying at its upper end a male screw of the R.M.S. standard diameter (effective) of 19·822 mm. (0·7804 in.) with 36 threads to the inch. This size has been universally adopted and thus objectives of all makes can be used on the same stand.

The lenses are screwed on the lower end of the mount, but some optical firms fit their objectives together in a manner which has much to recommend it. The lenses are no longer screwed the one on to the other, but are fixed into little metal cells which are then inserted into the hollow metal tube which constitutes the mount. This does away with small centring errors which might be introduced into the objective by the frequent screwing and unscrewing of its lenses during manufacture.

The mount is generally made of brass, lacquered or enamelled in black, the lower end being chromium-plated for high-power dry objectives and for immersions. Some firms employ a stainless white alloy which, should it prove really permanent after long periods, would present a decided advance over other materials and could be used with advantage for other non-enamelled parts of the microscope stand.

The lenses. The number of lenses varies from two or three in ordinary achromates to eight or more in apochromatic objectives. They may be simple or in systems of two or three elements cemented together with Canada balsam or some other substance

having the same refractive index as glass. According to the formula of the objective, many different combinations of lenses are used. In general, however, low-power objectives up to 8 mm. (1/3 in.) focal length are composed of two doublets or triplets, and the others of a simple hemispherical front-lens and two or three doublets or triplets.

Lenses are made of glasses having different refractive indices; principally flint-glass (R.I. 1·54—1·71) and crown-glass (R.I. 1·51—1·56). Semi-apochromatic and apochromatic objectives also contain a certain number of lenses made of fluorite, a mineral possessing a very low refractive index (1·4338) combined with a very low dispersion.

Classification of Objectives

According to their degree of chromatic correction, objectives are classed as *achromatic*, *semi-apochromatic* and *apochromatic*. According to the medium in contact with their front-lens, they are known as *dry*, *water-immersion* and *oil-immersion* (or *homogeneous immersion*) objectives. The latter are so named because the cedar-wood oil employed with them has the same refractive index as glass (1·54).

Nomenclature of Objectives

Objectives are designated by their focal length in millimetres or inches. Low-power dry achromatic objectives may sometimes be given arbitrary serial numbers or letters which differ from one maker to another except that the first numbers and letters of the alphabet correspond to the lowest powers.

When the focal length of an objective is known, its *initial magnification* can be calculated by dividing the number 250 (the distance of normal vision in millimetres) by the focal length also expressed in millimetres. Example: An objective of 50 mm. focal length would have an initial magnification of $250 \div 50 = 5$ linear diameters.

One sometimes reads in handbooks on optics that the *total* (or *combined*) *magnification* of a microscope can be found by multiplying the initial magnification of the objective by that of the eyepiece used. Some opticians' catalogues still employ this

calculation for the magnification tables of their objectives. In these tables the following figures may sometimes be found to indicate the magnification of an objective of, for example, 2 mm. (1/12 in.) focal length.

Initial magnification $250 \div 2 = 125$.

Total magnification with an ocular magnifying 10 diameters $125 \times 10 = 1250$.

However should one check this number with a stage micrometer and a camera lucida, one finds a total magnification of only 900!

This considerable discrepancy is due to the fact that the figure given as the initial magnification of the objective, indicates the magnification of the real image projected by it at a distance of *250 mm.* But the image which is received and subsequently magnified by the eyepiece is the real image formed by the objective in the *lower focal plane* of the eyepiece in question. This plane lies near the upper end of the drawtube and, as its distance from the objective is much smaller than 250 mm. (it varies around 160 mm. for modern objectives), the image received by the eyepiece has been less magnified in consequence.

In this connection, Zeiss has introduced a new term in microscopy, that of the *partial magnification* of an objective. The partial magnification gives the magnification of the image formed by the objective in the lower focal plane of the eyepiece. It is found by dividing the optical tube-length in millimetres (i.e. the distance between the upper focal plane of the objective and the lower focal plane of the eyepiece) by the focal length of the objective. This partial magnification of the objective multiplied by the initial magnification of the eyepiece gives the *true* total magnification of the microscope.

Zeiss has given up all other systems of nomenclature and the partial magnification, engraved on each objective, serves also to designate the latter. This new nomenclature is of great practical convenience as the true total magnification, a factor of importance to the microscopist, can be calculated immediately from it. Several other firms also give the partial magnification of each objective in their catalogues and use it as the basis of their magnification tables. It is to be hoped that all opticians

will some day follow suit so that greater uniformity may be introduced into this matter.

It is not easy to give any practical method for estimating the partial magnification as the optical tube-length varies from one objective to another, being in general shorter for the low than for the higher powers. The following formula will however give fairly accurate results:

For low-power objectives up to and including 25 mm. (1 in.) focal length, divide the figure 100 by the focal length in mm.

For medium-power objectives up to 6 mm. (1/3 in.) focal length inclusive, divide the figure 150 by the focal length in mm.

For high-power objectives divide the figure 200 by the focal length in mm.

Example: A 30 mm. (1 1/4 in.) F.L. objective will have an approximate partial magnification of $100 \div 30 = 3$ diameters.

A 3 mm. (1/8 in.) F.L. objective will have an approximate partial magnification of $200 \div 3 = 66$ diameters.

Finally any system of nomenclature will remain incomplete if no mention is made of the *numerical aperture*[1] of an objec-

[1] *Numerical Aperture.* Notation introduced by Prof. Abbe of Jena to define the separating power of an objective.

The need for such a notation made itself felt as soon as immersion objectives were invented, as the *angle of aperture* (the angle formed by the most oblique light rays which, diverging from the same point of an object, are capable of entering the front-lens of the objective) which is a sufficient criterion for dry objectives, does not permit a comparison between these and immersions. The reason for this difficulty is that the angle of aperture varies with the medium in contact with the front-lens of the objective considered. The numerical aperture does not present this disadvantage, and two objectives having the same numerical aperture also possess the same resolving power even if the one is a dry-lens and the other an immersion.

The numerical aperture of an objective depends on its angle of aperture and the medium in contact with its front-lens and is given by the formula $N.A. = n \sin u$.

'n' here represents the refractive index of the medium in contact with the front-lens, and 'u' the half of the angle of aperture of the objective *for the medium in contact with the front-lens.*

The notation thus obtained is applicable to all objectives as, for a given light cone, the second term of the above equation gives a *constant* number irrespective of the interposing medium. This is due to the fact that when the refractive index increases, the angle of aperture diminishes by a proportional amount and vice versa.

tive, as this is the only factor that can give full information on the fineness of detail which it is capable of revealing. An objective with a partial magnification of 90 and a numerical aperture of 1·25 will have much less resolving power than one of 1·40 N.A., even if the latter has a partial magnification of only 60.[1]

For these reasons all manufacturers should engrave on the mounts of each of their objectives: (1) The focal length in mm. (2) The numerical aperture. (3) The partial magnification. (4) A symbol showing whether the objective is achromatic, semi-apochromatic or apochromatic, such as the letters 'A', 'S.A.' and 'A.P.' It would be convenient too if the working distance (i.e. the free distance between the front-lens and the object examined, when the objective is sharply focused; in practice this generally means the distance between the front-lens and the upper face of the cover-glass), the mechanical tube-length and the cover-glass thickness for which the objective is corrected were marked on the objective case.

No serial number need be used, as the simplest way of designating an objective is to refer to it by its focal length expressed in millimetres.

Achromatic Objectives

Achromatic objectives are those in common everyday use which are corrected for only two spectral colours for the chromatic and for one for the spherical aberration. They consequently show traces of colour fringes when very delicate objects are being examined and this sometimes confuses the finer details. This fault becomes still more obvious when oblique light or dark-ground illumination is used. In photomicrography too, achromatic objectives are far inferior to the apochromates

[1] The number of lines per millimetre that can be resolved by an objective using *oblique illumination* is found by multiplying twice the numerical aperture by the frequency of the light employed.

The resolution of an objective using an *axial light cone* which fills 3/4 of its back-lens is found by multiplying the frequency of the light employed by 3/2 and again multiplying the result obtained by the numerical aperture. (Mr. E. M. Nelson, *Journal of the Royal Microscopical Society*, 1893.)

described further on. It is more difficult to bring them to a precise focus and, to obtain sufficiently sharp negatives, it is generally necessary to use them with colour-filters giving a more or less monochromatic light.

Apart from these disadvantages however, modern achromatic objectives by reputable makers give excellent images with nearly all classes of objects and are perfectly satisfactory except for the most exacting work.

The achromates most generally met with are enumerated in the following table which also gives their focal length, their partial magnification and their numerical aperture. These figures are, of course, only approximative as they vary slightly from one firm to another.

	Focal length		*Partial magnification for a mechan. tube-length of* 160 *mm.*	*N.A.*
	mm.	*in.*		
	75	3	1·5	—
	50	2	2	0·08
	40	1 2/3	2·5	0·10
	35	1 1/3	3	0·12
Dry	30	1 1/4	4	0·15
Objectives	25	1	5	0·20
	16	2/3	10	0·25
	12	1/2	15	0·32
	8	1/3	20	0·40
	6	1/4	25	0·65
	4	1/6	45	0·75
	3	1/8	60	0·80
Water-Imm.	2	1/12	90	1·15
	3	1/8	60	1·25
Oil-	2	1/12	90	1·30
Immersion	1·6	1/15	115	1.30
	1·3	1/16	125	1·30

Some opticians supply objectives up to 100 mm. (4 in.) F.L. with a partial magnification of less than 1. These objectives are convenient for studying the general aspect of a whole organ or of a large section. They are used more often in zoology and botany than in medicine.

Very high-power dry objectives are also manufactured, 2 mm.

focal length, partial magnification 100, numerical aperture 0·85, but they are not recommended for the reasons given further on (see page 35).

Apochromatic Objectives

In apochromatic objectives the chromatic aberration has been corrected for three colours of the spectrum and the spherical for two. They consequently give clearer and crisper images than the achromates and can be used with higher power oculars. These results are mostly due to the fact that some of their lenses are made of fluorite, a mineral with a very low dispersion and refractive index (1·4338).

Apochromates are also more luminous than the achromatics, but they have the disadvantage of a more curved field. This can be overcome to some extent by the use of special eyepieces such as Swift's 'Telaugic' or Watson's 'Holoscopic'. Incidentally, it should not be lost to mind that a large 'flat field' cannot be expected in a high-power objective; in fact the more perfect the lens the more obvious does the curvature become. In a good objective however every zone from the centre to the periphery of the field can be brought in succession into sharp focus by means of the fine adjustment.

Owing to the fact that apochromates are more particularly corrected for the violet end of the spectrum (under-correction), they cannot be used with ordinary Huyghenian eyepieces. Unless the special oculars such as those previously mentioned are employed, apochromatic objectives should always be used in conjunction with *compensating eyepieces* which neutralize, by their slight over-correction, the under-correction of the objectives.

The more usual apochromates are listed in the following table:

	Focal length in mm.	*Partial magnification. Mechanical T.L. 160 mm.*	*N.A.*
	16	10	0·30
Dry	8	20	0.60
Objectives	4	45	0·90
	3	60	0.95
Water-Imm.	2·5	70	1·25

	Focal length in mm.	*Partial magnification. Mechanical T.L. 160 m.m.*	*N.A.*
Oil-Immersion	3	60	1·30
	3	60	1·40
	2	90	1·30
	2	90	1·40
	1·5	125	1·30

Apochromatic objectives of 40, 25, 12 and 6 mm. with N.A.'s of 0·12, 0·25, 0·30 and 0·92 respectively are also made by Swift.

Comparison between Objectives of N.A. 1·40 and N.A. 1·30

The 2 mm. F.L. objective of 1·40 N.A. presents a gain of 8 per cent in resolution and 14 per cent in luminosity over that of 1·30.

On the other hand its front-lens is mounted on extremely narrow settings and can be easily displaced by a slight jar. Its working distance too is only half that of the other, or 0·05 mm.

As for the objective of only 3 mm. F.L. and 1·40 N.A., its low partial magnification of 60 makes high-power eyepieces necessary if all the detail resolved by its high numerical aperture is to be distinctly perceived. In the author's opinion at least, better results are obtained by using a 2 mm. F.L. objective (part. mag. 90) and a less powerful eyepiece.

To sum up: if the *very maximum* of resolving power is essential, the 2 mm. objective of 1·40 N.A. gives the best results. In all other cases the 2 mm. 1·30 N.A. objective is preferable owing to its greater working distance and less delicate front-lens.

Semi-Apochromatic Objectives

Owing to their complexity and to the rarity of good quality fluorite, apochromates are very expensive. For this reason many firms produce simpler objectives containing a smaller number of fluorite lenses. These objectives, which stand midway between apochromates and achromates, are known as semi-apochromatic or fluorite objectives. They can be used with Huyghenian or, preferably, with compensating oculars.

The semi-apochromatic formulae are generally reserved for high-power dry or immersion objectives, most often the following:

	Focal length		*Partial magnification.*	*N.A.*
	mm.	*in.*	*Mechanical T.L. 160 mm.*	
	4	1/6	45	0·80
Dry	3	1/8	60	0·85
Objectives	2·5	1/10	70	0·85
	2	1/12	90	0·90
	3·5	1/7	50	0·95
Oil-	2	1/12	90	1·30
Immersion	1·6	1/15	115	1·30
	1·5	1/16	125	1·30

Cover-Glass Thickness and Mechanical Tube-Length

To get the best results from an objective, it must be used with the exact cover-glass thickness and mechanical tube-length for which it has been corrected by the manufacturers.

Cover-glass thickness. Most optical firms correct their objectives for a cover-glass thickness of 0·17 or 0·18 mm. It is very important to stick to the right thickness so as not to upset the objective's correction for spherical aberration. Fortunately, as described later (page 85), variations in cover-glass thickness can be neutralized by means of the drawtube or by the use of objectives provided with a correction collar.

Mechanical tube-length. All objectives are corrected for a certain fixed tube-length. This length, known as the *mechanical tube-length*, is measured from the lower end of the body-tube, where the objective is screwed, to the upper end of the drawtube which supports the rim of the eyepiece. This length is not the same for all manufacturers. Most have adopted a length of 160 mm., but some (Watson, Leitz, Seibert) correct their objectives for a tube-length of 170 mm.

In the author's opinion, all opticians would do well to adopt a tube-length of *180 mm.* for the following reasons:

It will be found in practice that the cover-glass thickness used is generally far greater than that for which the objective has been corrected as it is necessary to take into consideration not only the thickness of the cover-glass itself, but also that of the object examined and the medium in which it is mounted. To remedy this, it will be necessary to *shorten* the drawtube, perhaps

to a considerable extent. It will be seen at once however that, if the objective has been corrected for a mechanical tube-length of 160 mm. and the microscope body-tube is 150 to 155 mm. long (as is generally the case), the drawtube cannot sometimes be shortened enough to obtain the sharpest image that the objective is capable of giving. In other words, for many classes of work, such as histology, pathological anatomy, etc., where thick sections are often studied, *it may be materially impossible to see the microscope image with its maximum clearness.* This drawback would not exist if all objectives were corrected for a tube-length of 180 mm. because there would then be more latitude either way for the necessary adjustments, since on most stands the drawtube can be shortened to about 150 and lengthened to at least 200 mm.

The Correction Collar

Objectives having a low numerical aperture show very little sensitivity to different cover-glass thicknesses. This is no longer the case when objectives of 0·50 N.A. and more are reached, and for this reason high-power dry objectives and water-immersions are sometimes provided with a correction collar. This accessory is not needed in oil-immersions which are not much influenced by variations in the cover-glass thickness.

The correction collar is a metallic ring, encircling the upper part of the objective mount, by means of which the relative positions of certain of the inner elements can be altered to counteract the spherical aberration introduced by the cover-glass. It carries divisions corresponding to cover-glass thicknesses of 0·1 to 0·2 mm. and, by bringing these numbers opposite a stationary pointer, the objective is automatically corrected for the thickness indicated. It goes without saying that, even if the exact cover-glass thickness were known beforehand, these divisions would only give an approximative result as the thickness of the medium in which the object is mounted would not have been taken into account. The scale is consequently of limited practical value and the best way of setting the correction collar is by observing the microscope image as described on page 90.

Correction collars raise the price of objectives considerably,

often by more than a third. They have also the disadvantage of making the accurate centring of the various elements more difficult and for this reason many optical firms have ceased their manufacture, leaving it to the microscopist to effect the corrections by means of the drawtube. This would perhaps be the best solution to the problem if objectives were corrected for a tube-length of 180 mm. which would leave a greater latitude for the necessary adjustments.

Dry Objectives

As their name implies, dry objectives are those whose front-lens is in contact with no other medium than the air. They were the first in line of discovery as the compound microscope seems to have been invented between 1590 and 1609 by the spectacle-makers Hans and Zacharias Janssen or Hans Lippershay at Middelburg in Holland.

Dry objectives are the handiest to use provided that those having a smaller focal length than 4 mm. (1/6 in.) are avoided. Dry objectives with a very small focal length have such a short working distance that it is often impossible to focus a fairly thick object without the front-lens touching the cover-glass. These objectives also have a very low luminosity and a very curved field; for high powers consequently it is far better to choose immersion objectives.

Water-Immersion Objectives

Water-immersion objectives are those whose front-lens is in contact with distilled water which fills all the space between it and the cover-glass. They were invented in 1815 by Professor Amici of Modena and perfected later by Hartnack and Prazmowski of Paris.

After having enjoyed a great vogue, water-immersions are now little used and many optical firms have ceased to make them. It is a pity, in our opinion, that these objectives have been so much neglected as they present certain valuable advantages over the oil-immersion.

In the first place, when studying objects immersed in a liquid of low viscosity (water, serum, etc.), it often happens, when using

an oil-immersion, that the cover-glass adheres more strongly to the objective front-lens than to the slide owing to the higher viscosity of the cedar-wood oil. The cover-glass is thus held back and displaced when the object-slip is moved forward and much trouble and loss of time is the result.

Another advantage, when a great number of slides have to be studied, is that it is unnecessary to clean each object-slip after examination as the distilled water evaporates without leaving any trace. The drawback of forgetting to wipe the cedar-wood oil from the objective front-lens after work is also eliminated.

Finally, for most laboratory routine work, there is little to choose between water- and oil-immersions as far as definition and resolution are concerned, especially when used in conjunction with such a poor illuminator as the Abbe condenser. With the latter, the effective numerical aperture (see page 41) is 0·82 for the water- and 0·90 for the oil-immersion, that is to say the possibility of resolving 31,124 lines per centimetre against 34,161 in white light. This small difference would not be appreciable in ordinary work.

Oil or Homogeneous Immersion Objectives

With oil-immersion objectives cedar-wood oil is used to fill the space between the front-lens and the cover-glass. They are also known as *homogeneous* immersions because the cedar-wood oil has approximately the same refractive index as glass (1·515) and consequently the objective and the cover-glass constitute one whole from the optical point of view.

Oil-immersions were the last to be invented. From Amici onwards, opticians had foreseen the advantages to be gained from an immersing fluid with a higher refractive index than that of water. Various liquids were tried at different times and, in 1876, Tolles of Boston had experimented with Canada balsam as an immersing medium. Finally, following the suggestions of H. Stephenson of London, Professor Ernst Abbe of Jena began, in 1876, his famous researches which resulted in the cedar-wood oil-immersion objective as we know it to-day.

Oil-immersions are far superior to dry objectives, as they can

be given much higher numerical apertures. An oil-immersion presents too, over a dry objective of the same numerical aperture, the advantages of a longer working distance, and a greater luminosity owing to the fact that no rays are lost by reflection from the anterior surface of the front-lens.

Limit to the Useful Magnification of Objectives

It is possible to prove, by starting from the number of lines per centimetre resolvable in white light by the naked eye, that a magnification of about 540 diameters is sufficient to show all the detail that an objective of 1·40 N.A. is capable of revealing. (Under the best conditions, the normal eye can resolve about 98 lines per centimetre in white light. An objective of 1·40 N.A. can resolve 53,139. If this is divided by 98, the figure 540 is found.) This means that, by increasing the magnification beyond this limit, the details already visible are still further enlarged, but no *new* ones are shown. This is known as *empty magnification.* To see a detail however without strain or fatigue, the eye generally needs a somewhat higher magnification than the theoretical one and in practice this can with advantage be doubled. It is usually admitted that the limit of useful magnification, beyond which no advantage is derived, is one thousand times the numerical aperture of an objective.

Example: For an objective of 0·30 N.A. the maximum limit of useful magnification is 300 diameters; for one of 1·25 N.A. it is 1,250 diameters.

Optical Index

From what has already been said, it is clear that there is a very close relation between the numerical aperture of an objective and its useful magnification. It is evident that if a magnification of 540 is sufficient to show to a skilled eye all the detail that an objective of 1·40 N.A. is capable of revealing, then theoretically every objective should have a numerical aperture of 0·26 for each magnification of 100 diameters.

$$1{\cdot}40 \div 540 = 0{\cdot}0026$$
$$0{\cdot}0026 \times 100 = 0{\cdot}26$$

In other words, an objective of 0·26 N.A. magnifying 100 times or one of 0·52 N.A. magnifying 200 times (combined magnification with the eyepiece) shows to a skilled eye all the detail it is capable of revealing. An objective of 4 mm. (1/6 in.) F.L. having a N.A. of only 0·26 and a combined magnification of 400 with the × 10 ocular, would be a very bad objective as most of its magnifying power would only amplify details already resolved without showing any new ones (empty magnification).

Mr. E. M. Nelson of London has accordingly introduced a new formula for determining the optical value of an objective. It consists of multiplying the numerical aperture by 1,000 and dividing the result by the initial magnification of the objective. The quotient thus obtained is a figure which Mr. Nelson has named the *optical index*. The closer its optical index is to 26, the greater is the optical value of an objective. The ideal objective would have an optical index of exactly 26.

Thus, if we again take the 4 mm. objective of 0·26 N.A. already used as an example and compare it with one of 16 mm. (2/3 in.) F.L. and the same N.A., the advantage remains with the second lens. By making the calculations we find:

For the first objective (initial magnification 62·5).

$$\frac{0{\cdot}26 \times 1000}{62{\cdot}5} = \frac{260}{62{\cdot}5} = 4{\cdot}16$$

For the second objective (initial magnification 15·6).

$$\frac{0{\cdot}26 \times 1000}{15{\cdot}6} = \frac{260}{15{\cdot}6} = 16{\cdot}66$$

This shows that the optical index is 16·66 for the 16 mm. objective while it is only 4·16 for that of 4 mm.

2. THE EYEPIECE OR OCULAR

Eyepieces can be divided into three large groups: *Huyghenian eyepieces*, the ordinary oculars used with achromatic objectives. *Compensating eyepieces*, used with apochromates. *Special eyepieces*, used for certain specific purposes.

Huyghenian Eyepieces

These eyepieces, composed of two lenses with their convex faces turned towards the objective, are employed with ordinary achromatic objectives. They are made in different powers and are generally numbered by their makers with Arabic or Roman numerals. These serial numbers are purely arbitrary and do not correspond from one optician to another; consequently it would be very convenient if all firms were to mark each ocular with its initial magnification and its focal length in millimetres. Some manufacturers already do this.

The commonest Huyghenian eyepieces are the following:

Focal length in mm.	*Initial magnification*
75	3
50	5
36	7
25	10
20	12
17	15

The most useful oculars are those of 50 mm. F.L. × 5 and 25 mm. × 10.

Compensating Eyepieces

Compensating eyepieces incorporate a system of lenses designed to give a certain degree of over-correction and thus annul the under-correction present in all apochromatic objectives. It is worth while noting that semi-apochromates and high power achromatic objectives from 4 mm. (1/6 in.) F.L. are also somewhat under-corrected and consequently give better images when used with compensating oculars.

Compensating eyepieces are generally distinguished by an engraved number expressing their initial magnification; with some firms however these numbers do not correspond very closely with the true initial magnifications.

The more usual compensating eyepieces are given in the table below:

Focal length in mm.	*Initial magnification*
83	3
50	5

Focal length in m.m.	*Initial magnification*
36	7
25	10
17	15
12·5	20

The most generally useful oculars are the 50 mm. F.L. × 5 and the 25 mm. × 10.

Special Eyepieces

This class of eyepiece includes:

Oculars provided with a short drawtube to adjust their degree of correction. This allows them to be employed both with achromates and apochromates and makes it unnecessary to have two series of eyepieces for the two types of objective. (Watson's 'Holoscopic' oculars.)

Eyepieces designed to reduce as much as possible the curved field of high-power objectives, especially the apochromates. (For apochromates; Swift's 'Telaugic compensating', Leitz's 'Periplanetic'. For achromates: Baker's 'Gifford orthochromatic', Swift's 'Telaugic', Bausch and Lomb's 'Hyperplane', Nachet's 'Planachromatic', Spencer Lens Company's 'Planoscopic', etc.)

Eyepieces for photomicrography. (Projection eyepieces, Zeiss's 'Homals', etc.)

Miscellaneous eyepieces for various work. (Micrometer, spectroscopic, demonstration, comparator, drawing, binocular, angle eyepieces, etc.)

Diameter of Eyepieces

The eyepiece most usually seen is the R.M.S. Universal small or student's size to fit a drawtube having an internal diameter of 23·3 mm. (0·9173 in.). A large or capped size is also made for a drawtube of 32·26 mm. (1·27 in.) internal diameter.

Incidentally, it may be pointed out here that the eyepieces with the shorter mounts are the more powerful; the inverse is true of objectives.

Should there be any doubt as to whether an ocular is Huyghenian or compensating, it should be looked through and the

colour around the margin of the field noted. If this is blue the eyepiece is Huyghenian, if orange it is compensating.

3. THE CONDENSER

Condensers fall into two principal categories: *light-ground* or transmitted light condensers and *dark-ground* or diffracted light condensers.

Light-Ground Condensers

Light-ground condensers are made in two forms: *Dry condensers* and *immersion condensers*. A good condenser is almost as carefully corrected as an objective and the same one cannot be used indifferently dry or immersed without sacrificing some of its optical qualities.

Dry Condensers. Dry condensers can be divided into two classes: ordinary condensers of the *Abbe* type which are neither achromatic nor aplanatic, and *achromatic and aplanatic* condensers.

The *Abbe* condenser was invented by Professor Abbe in 1872 and is still one of the most popular owing to its low price and the ease with which it can be used. It is made in two patterns, the first composed of two lenses and having a N.A. of 1·20 and the second of three lenses with a N.A. of 1·40. (These figures represent the condenser's total numerical aperture when its front-lens is united to the slide with a drop of cedar-wood oil. The total numerical aperture of a *dry* condenser cannot surpass 1·0.)

Unfortunately the above figures only give the *total* numerical aperture and the Abbe condenser is so poorly corrected that all the light does not converge to the same spot, but the oblique rays have a shorter focus than the central ones (Fig. 3A). For this reason it is impossible to make use of the total numerical aperture and the *aplanatic* numerical aperture, which can alone be employed, is little more than 0·50 for both patterns, even when immersed.

It is consequently evident that, although the Abbe condenser is sufficient for objectives whose numerical aperture is not greater than about 0·70, it cannot be used with objectives of

greater numerical aperture without sacrificing many of their advantages. The *effective numerical aperture* of an objective is found by adding its numerical aperture to the *aplanatic* numerical aperture of the condenser and dividing the total by two.

An Abbe condenser used in conjunction with an objective of 1·30 N.A. reduces the latter to 0·90.

$1{\cdot}30 + 0{\cdot}50 = 1{\cdot}80 \qquad 1{\cdot}80 \div 2 = 0{\cdot}90$

Thirty per cent of the objective's resolving power is thus wasted.

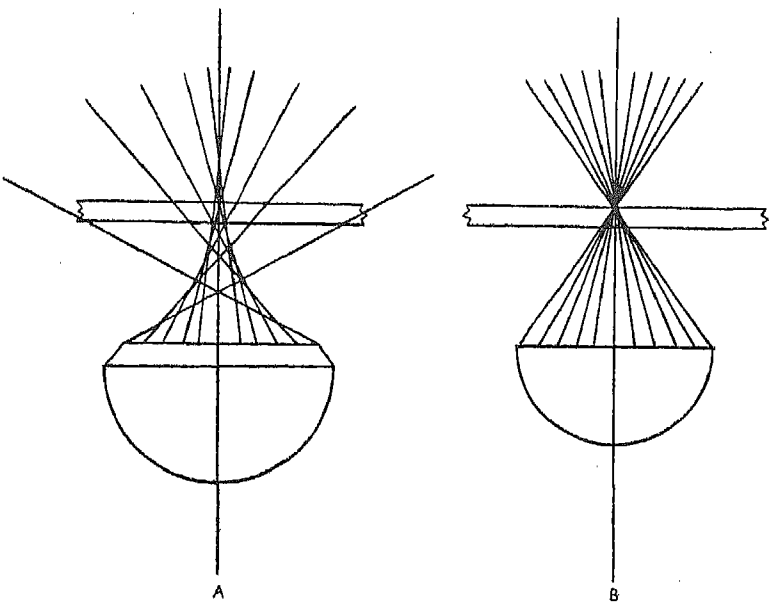

FIG. 3

This is an important point to remember, as we have ourselves seen persons utilizing an Abbe condenser with apochromatic objectives when they would have obtained far better results, at considerably less expense, with ordinary achromates and a good achromatic and aplanatic condenser. With a dry achromatic condenser, for example, having an aplanatic numerical aperture of 0·95 the same objective of 1·30 N.A. would give an effective numerical aperture of 1·125 instead of only 0·90.

$1{\cdot}30 + 0{\cdot}95 = 2{\cdot}{\cdot}25 \qquad 2{\cdot}25 \div 2 = 1{\cdot}125$

The *achromatic and aplanatic condenser*. The ordinary non-achromatic condenser is not suitable for getting an objective of high numerical aperture to perform at its best. The finest objective cannot be expected to give an image free of all colour fringes if the cone of light it receives from the condenser is itself badly corrected chromatically. An achromatic condenser is consequently indispensable for the best results. In addition to this, as has already been pointed out, the aplanatic numerical aperture of the condenser must not be very much smaller than that of the objective with which it is to be used. This implies good correction of the spherical aberration.

For these reasons, manufacturers have produced condensers whose corrections rival those of the best objectives. These condensers, known as *achromatic and aplanatic* or more simply as *achromatic condensers*, are generally composed of five lenses. They focus all the light rays at exactly the same spot and thus give a perfectly sharp and achromatic image of the light source (Fig. 3B). Owing to these improvements, their aplanatic numerical aperture can be as high as 0·95 or even more, their total numerical aperture being in the neighbourhood of 1·0.

Achromatic condensers are very sensitive to slide thickness and only work at their best if the thickness is that for which they have been corrected. Most firms correct their condensers for a slide thickness of 1·5 mm. This subject is further treated on page 79.

Some Continental opticians supply a condenser which stands about midway between the Abbe and the achromatic condenser; this is the aplanatic three lens Abbe condenser. This type is not achromatic however and must be used with monochromatic light to give satisfactory results. It is consequently inferior to the achromatic and aplanatic condenser.

Immersion Condensers. The numerical aperture of a dry condenser cannot be greater than 1·0 and consequently, to surpass this limit, the front-lens of the condenser must be united to the under surface of the object-slip with a drop of cedar-wood oil. This has been realized in the *immersion* achromatic and aplanatic condensers. These condensers, mostly composed of six lenses, have an aplanatic numerical aperture of 1·30 to 1·40 and thus

allow the whole of the numerical aperture of immersion objectives to be utilized.

Immersion condensers can also be employed dry, but in that case their maximum numerical aperture cannot naturally surpass 1·0 and their performance is slightly less satisfactory since their formulae have been computed for immersion use. They have a very short focus and cannot generally be utilized with slides thicker than 1·5 mm. for the 1·30 N.A. or 1·0—1·3 mm. for the 1·40 N.A. condensers.

All condensers, whatever their type, project in the plane of the object examined a very much reduced image of the light source. This makes it difficult sometimes to fill the whole field of a low-power objective with light if a very short focus condenser is used. This difficulty can be overcome to some extent by removing the front-lens of the condenser which will then give a less bright but larger image of the illuminant. Some firms also supply condensers with an extra long focal length specially made for very low-power objectives (Baker's 'Nelson', Swift's 'Paralux', Watson's 'Macro-illuminator', etc.).

The Iris Diaphragm

Every condenser should be provided with an iris diaphragm forming an integral part of its mount. The plane of this iris should coincide with the condenser's lower focal plane and its centre with the condenser's optical axis as it is by observing the iris opening that the condenser can be centred to the optical axis of the objective by means of the centring screws.

It is very convenient if the slot along which the iris lever moves can be divided so as to indicate the numerical aperture at which the condenser is being used for a given opening of the diaphragm. If this has not already been done, it can be achieved as follows: An objective of known numerical aperture, say 0·20 N.A., is screwed on to the microscope and both it and the condenser are focused on some detail of the object-slip as described on page 77. The eyepiece is removed and the back-lens of the objective examined by looking down the drawtube. The iris is slowly closed until its shadow just appears around the periphery of the back-lens and a small mark is then made on the condenser

mount at the position now occupied by the iris lever. This mark will indicate in future the iris opening which reduces the numerical aperture of the condenser to 0·20. The same process is repeated with objectives of gradually increasing power until the iris has been calibrated for all numerical apertures.

The condenser should also be provided, beneath the iris diaphragm, with a carrier for stops and coloured filters. A revolving carrier is very useful if work with oblique illumination is contemplated.

Dry and Immersion Condensers Compared

Experience has shown that no objective, however high its quality, can stand a light cone equal to its own full numerical aperture without a serious deterioration of the image. Most good objectives can only stand a cone equal to 3/4 of their numerical aperture. Some few—among apochromates only—can stand a cone of 4/5 of their numerical aperture and a minority of quite exceptional lenses a 5/6 cone.

The 3/4 of a numerical aperture of 1·40 is only 1·05 and of a numerical aperture of 1·30 is 0·97. It would therefore seem as if a dry condenser, whose numerical aperture is almost 1·0, could supply the largest light cone that any objective can stand without breaking down.

Unfortunately dry condensers have certain disadvantages. They are very sensitive to slide thickness and if this differs even slightly from that for which they have been corrected, their optical performance shows a distinct falling off. This means that the condenser must be corrected anew each time the slide is changed (see page 79), a manipulation which is unnecessary with immersion condensers as these are connected to the slide by a liquid having the same refractive index as glass.

Owing to their higher numerical aperture and to the fact that no light is lost by reflection from the under surface of the slide, immersion condensers pass more light than dry ones. This is a valuable advantage for high power work and especially for photomicrography. Finally, if one should be fortunate enough to possess one of those exceptional objectives of 1·40 N.A. which can stand a 5/6 cone (i.e. a cone of 1·16 N.A.), the in-

crease in resolving power conferred by an immersion condenser is not to be disdained. It goes without saying that an immersion condenser of 1·40 N.A. can only work at its full numerical aperture when the object to be examined is mounted in a medium whose refractive index is equal or superior to that same figure, such as Canada balsam, cedar-wood oil, etc. The maximum numerical aperture of a condenser will only be 1·33 for an object mounted in water or serum and 1·0 for a dry preparation with air between the slide and the cover-glass.

Immersion condensers have the disadvantage of making it necessary, after use, to clean the cedar-wood oil from the front-lens and from the slides. The immersion liquid also shows a disagreeable tendency to seep between the object-slip and the stage or to trickle down the condenser mount. These inconveniences however are less important than the many advantages of the immersion condenser for the more exacting classes of work. The 1·30 N.A. model is generally to be preferred as it can supply the full illuminating cone that any objective, even one of 1·40 N.A. will stand and has a greater focal length than the other pattern.

Dark-Ground Condensers

Dark-ground condensers are sometimes erroneously termed 'ultra-microscopes', an appellation which should be reserved for the lateral illuminating apparatus of Siedentopf and Zsigmondy. For optical reasons, *all* dark-ground condensers are immersion condensers, none can be used dry. Most of them employ cedar-wood oil as the immersion fluid, but some models give good results with golden syrup, glycerine or distilled water.

Dark-ground condensers belong to two principal types. *Paraboloid condensers* and *spherical surface* (or *concentric*) *condensers.*

With the paraboloid pattern larger cones are obtained. Watson's 'Cassegrain' Dark-Ground Illuminator allows the full numerical aperture of an objective of 1·40 N.A. to be utilized if the refractive index of the medium in which the object is mounted is greater than 1·42.

Concentric condensers give, on the whole, crisper and brighter images. Zeiss's Luminous Spot-ring Condenser allows

the full aperture of a 1·30 N.A. objective to be used and Beck's Patent Focusing Dark Ground Illuminator one of 1·20 N.A. Moreover the latter, as its name implies, has an adjusting mechanism which allows it to be focused through slides 0·5 to 1·5 mm. thick. With 1·5 mm. slides an objective of 1·0 N.A. can be used at full aperture, and with 0·5 mm. slides one of 1·20 N.A.

Ordinary routine work dark-ground condensers cannot give a perfectly black background with objectives of more than 0·90 N.A. They must consequently be used with objectives of that numerical aperture or less. Higher numerical apertures must be cut down by a suitable stop (funnel stop) placed as close as possible to the objective back-lens.

Diameter of the Condenser Mount

The mount of all condensers, whether light- or dark-ground, should have an external diameter which will allow it to fit, neither too tightly or too loosely, into the R.M.S. standard size substage of 38·786 mm. (1·527 in.) internal diameter.

CHAPTER III

CHOICE OF A MICROSCOPE. TESTS FOR THE MICROSCOPE

CHOICE OF A MICROSCOPE

When choosing a microscope for any type of work there are three rules which can only be neglected at the purchaser's peril: The instrument must be by a reputable firm. It must be a fairly recent model. It must be *new*. Except under special circumstances, it is risky to buy a second-hand microscope unless one has a thorough knowledge of what qualities and defects should be looked for.

It is best to choose a microscope with a black enamel finish as this is more resistant than lacquered brass. Certain enamels are even impervious to the action of alcohol and acids which remove most lacquers. One should always insist on a microscope with the standard R.M.S. eyepiece, objective and substage diameters mentioned in the previous chapters.

Three types of microscope will be described: (1) Small sized microscope for students or for low-power work. (2) Medium or large sized microscope for advanced students or medical practitioners and for routine laboratory work. (3) Research microscope for critical work and for photomicrography.

1. SMALL SIZED MICROSCOPE FOR STUDENTS OR FOR LOW-POWER WORK

It cannot be stressed too strongly that it is better to buy from the outset a medium or large sized stand, even if this means reducing the optical outfit as this can always be completed later. The smaller stands are not to be despised however and they can prove of the utmost value to those whose means are limited or who only intend to do low-power work in certain

branches of histology, parasitology, pharmacology, zoology or botany.

The first question to be decided, when buying a small stand, is whether it is preferable to choose a model which has only a rack and pinion coarse adjustment or one that has some form of fine adjustment and a sliding tube for rapid focusing. There is little doubt that, if the work contemplated does not require magnifications exceeding 400 diameters, the first model should be chosen without hesitation. The second form, except in particularly skilful hands, is generally the cause of so much loss of time and inconvenience that it should be avoided. If higher magnifications are needed, then it is imperative to select a larger stand provided with both focusing mechanisms.

This question having been decided, the points of a cheap but effective microscope, one reduced as it were to its bare essentials, can be summarized as follows:

Stand

(a) Non inclinable foot and limb.

(b) Well made coarse adjustment by diagonal rack and pinion.

(c) Body-tube taking objectives of the standard R.M.S. thread and provided with a drawtube giving a mechanical tube-length of about 140 to 180 mm. This drawtube need not bear a millimetre scale, but an engraved line should show the 160 mm. tube-length for which most objectives are corrected. If objectives of not less than 12 mm. (1/2 in.) focal length are to be used, the drawtube can at a pinch be dispensed with, but it will be found invaluable with higher powers. The modern tendency to treat the drawtube as an unimportant extra in all small stands is a great mistake in our opinion. It is very convenient, as mentioned in Chapter I, if the lower end of the drawtube is fitted with a standard R.M.S. objective thread for very low powers, and its eyepiece end should, of course, have the standard inside diameter of 23·3 mm.

(d) Fixed square stage measuring at least 9 cm. × 9 cm. (3 5/8 in. × 3 5/8 in.) with two spring-clips.

(e) Plain tube substage of the standard R.M.S. inner diameter of 38·786 mm.

Optical Outfit

(a) *Objectives:* Achromates of 16 mm. (2/3 in.) and 4 mm. (1/6 in.) focal length with reduced numerical aperture, about 0·18 N.A. for the former and 0·65 for the latter, are perhaps the most useful objectives for the medical student. In some types of work, such as freshwater biology for instance, the 6 mm. (1/4 in.) will probably be preferable to the 4 mm. objective owing to its longer working distance and greater depth of focus. If only low-power work is to be undertaken, the 35 mm. (1 1/3 in.) and the 8 mm. (1/3 in.) objectives will be found very suitable.

(b) *Eyepieces:* A Huyghenian ocular of 50 mm. focal length × 5 is the first choice. After that, one of 25 mm. × 10.

(c) *Condenser:* The ordinary two lens Abbe condenser with iris diaphragm and stop carrier will be found satisfactory enough. For low-power objectives of not less than 12 mm. (1/2 in.) focal length the condenser may even be omitted. The plain tube substage can then be replaced by a revolving disc diaphragm and the plane mirror by a concave one.

Accessories

The most important accessory is a revolving double nosepiece.

2. MEDIUM OR LARGE SIZED MICROSCOPE FOR ADVANCED STUDENTS OR MEDICAL PRACTITIONERS AND FOR ROUTINE LABORATORY WORK

A. MEDIUM SIZED MICROSCOPE

Stand

(a) Foot and limb with inclining axis.

(b) Coarse adjustment by diagonal rack and pinion.

(c) Fine adjustment of modern type, preferably by micrometer screw and horizontal or vertical lever.

(d) Body-tube with graduated drawtube giving mechanical tube-lengths of about 140 to 200 mm. Chromium-plated or enamelled drawtube with cloth-lined sleeve. Diaphragm at lower end of drawtube with a free diameter of at least 1·75 cm. (11/16 in.) and fitted with a standard R.M.S. thread for very

low-power objectives. The drawtube should be slightly larger at about 2·5 cm. (1 in.) from its upper end to prevent polishing by long eyepieces. The inside of body and drawtube should be dead-black and they should conform to the standard R.M.S. gauges at objective and eyepiece ends.

(e) Double or triple nosepiece—preferably the latter.

(f) Square stage about 10 cm. × 10 cm. (4 in. × 4 in.) covered with ebonite or some similar substance. An attachable mechanical stage is a very important accessory.

(g) Screw focusing substage of standard R.M.S. diameter *with centring screws.*

(h) Double mirror 5 cm. (2 in.) in diameter.

Optical Outfit

(a) *Objectives:* Achromates of 16 mm. (2/3 in.) and 4 mm. (1/6 in.) focal length. The latter should preferably be of reduced numerical aperture—about 0·75—so as to have a sufficient working distance to focus through haemacytometers, etc., 2 mm. (1/12 in.) oil-immersion achromate or semi-apochromate of not more than 1·30 N.A.

(b) *Eyepieces:* Huyghenian oculars of 50 and 25 mm. F.L. × 5 and × 10.

If it can be afforded, a 8 mm. (1/3 in.) objective will be found very useful; also a 25 mm. compensating eyepiece × 10 to employ with the 4 mm. objective and the immersion.

(c) *Condenser:* Ordinary two lens Abbe—or better still a dry achromatic condenser—with iris diaphragm and stop carrier.

Concentric condenser to take the place of the Abbe for dark-ground work.

B. LARGE SIZED MICROSCOPE

Stand

In all its essential parts, the stand will be the same as the one just described. The only difference would reside in its larger size, its *built-in* mechanical stage and its rack and pinion focusing substage.

Optical Outfit

(a) *Objectives:* As previously described, except that part or

all of the achromates may be replaced by semi-apochromatic objectives (Watson's 'Holoscopic' objectives are placed in this series). If the work does not require the maximum of resolving power, the oil-immersion may be replaced by a water-immersion whose advantages have been described on page 35. This also holds good for the medium-sized stand.

(b) *Eyepieces:* As for the medium stand. The 50 mm. × 5 compensating ocular might also be added.

(c) *Condensers:* Achromatic dry condenser and concentric dark-ground condenser.

3. RESEARCH MICROSCOPE FOR CRITICAL WORK AND PHOTOMICROGRAPHY.

Stand

The stand would resemble the previous one, with the following additions: Body-tube of large diameter, 4—5 cm. (1 3/4—2 in.), with an iris diaphragm mounted above the nosepiece. The latter is very useful for reducing the numerical aperture of an objective when greater depth of focus is required and in dark-ground work. Mechanical drawtube as described on page 15. Built-in mechanical stage of the pattern described on page 19. Rotating stage with centring screws and clamp. Fine adjustment to the rack and pinion focusing substage. Stainless steel plane mirror.

Optical Outfit

(a) *Objectives:* Some or all of the achromatic objectives could with advantage be replaced by apochromates. If a portion only are replaced, the best selection would perhaps be the following: 16 mm. (2/3 in.) F.L. achromate; 8 mm. (1/3 in.) apochromate; 4 mm. (1/6 in.) achromate with long working distance; 2 mm. (1/12 in.) oil-immersion apochromate of 1·30 or 1·40 N.A. For the advantages and disadvantages of these two objectives see page 32.

(b) *Eyepieces:* If the outfit is composed of apochromates only, the most useful oculars are those of 50, 25 and 17 mm. F.L. compensating with initial magnifications of 5, 10 and 15 diameters respectively. If both achromates and apochromates

are used, two Huyghenian eyepieces of 50 and 25 mm. F.L. would have to be added, or the two series of eyepieces could be replaced by Watson's 'Holoscopic' oculars which are suitable for both types of objectives.

(c) *Condensers:* Achromatic and aplanatic immersion condenser of 1·30 N.A. Concentric dark-ground condenser.

TESTS FOR THE MICROSCOPE

A *new* stand from a reputable firm can generally be relied upon for quality, but it is well worth while spending a little time on a few simple tests as the worker is thus familiarized with his own microscope. No second-hand instrument should however be bought without subjecting it carefully to the following tests. These include: (1) Tests for the stand. (2) Tests for the optical system.

It goes without saying that only those with some experience of the microscope should attempt these tests as otherwise they may be unfair to the instrument or to their own pockets.

1. TESTS FOR THE STAND

The Foot

Make sure that the microscope is absolutely steady when inclined to the horizontal for the tripod, or to an angle of at least 30 degrees for the horseshoe model. (With the latter the instrument can be steadied by fixing the foot to a base-board or by placing a weight across the front ends of the horseshoe on the rare occasions—except in photomicrography—when the horizontal position may be necessary.) Reject any stand whose foot has more than three points of contact with the table. The inclining axis should be neither too stiff nor too loose, and should be provided with an adjusting spanner or a built-in clamping lever.

The Limb

There should be a free space of at least 7·5 cm. (3 in.) between the base of the limb and the stage aperture. Preference should always be given to the pattern in which the limb is prolonged beneath the stage like an optical bench to carry the substage fitting.

The Body

Make sure that the eyepieces fit easily, but without too much play, in the upper end of the drawtube and that the latter slides smoothly in its sleeve without however sinking from its own weight when the microscope is in a vertical position.

The finger should be passed down inside the drawtube to ascertain if there is a slight increase in its diameter a few centimetres from the top to prevent the inside surface from being polished by low-power eyepieces.

Note if the body and the lower end of the drawtube are threaded to take objectives of the standard R.M.S. size and if

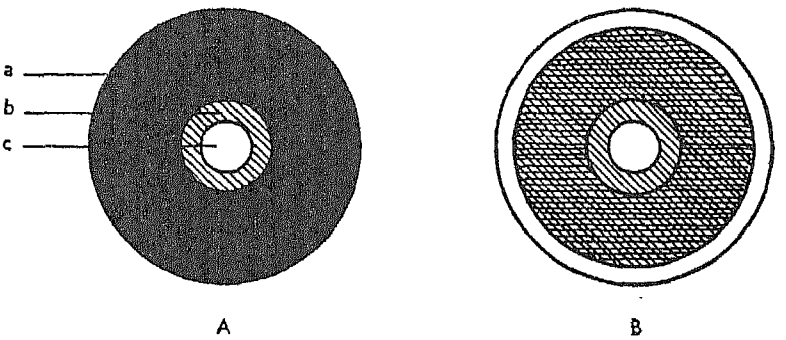

FIG. 4

the diaphragm at the bottom of the drawtube has a free diameter of at least 1·75 cm. (11/16 in.).

Carry out the following test: With a 4 mm. (1/6 in.) objective and a × 10 eyepiece, focus any suitable object, using transmitted light (see page 74). Open the condenser iris completely, remove the eyepiece and, looking down the microscope tube, slowly close the iris until only 3/4 of the objective back-lens is filled with light.

Replace the ocular and examine the Ramsden's circle (i.e. the small bright patch of light which is seen just above the top-lens of the eyepiece) while holding the eye about 15 cm. (6 in.) distant from the ocular in question. One should observe the aspect represented at 'A' of Fig. 4.

The large broad black circle 'a' is the top-lens of the eyepiece,

the little greyish concentric circle 'b' is the image of the objective back-lens and the bright central disc 'c' is the opening of the condenser iris. The circle 'a' must be completely and evenly black as in 'A'. If it is only grey, if it presents light and dark areas or a bright outer zone as in 'B', it means that stray reflections are entering the eyepiece to spoil the optical perfection of the image.

This defect—less frequent in stands with a body of large diameter—is caused by light rays which are reflected from the inner surface of the body-tube and enter the eyepiece without having been stopped by the diaphragm at the lower end of the drawtube. The reflected light which thus passes through the eyepiece outside the Ramsden's circle is generally weak, but it is often quite sufficient to make the image less crisp and to diminish contrasts in photomicrography, especially with long exposures.

Mr. E. M. Nelson has described an easy way to correct this fault. A disk of blackened cardboard is cut out, of the same diameter as the inside of the drawtube, with a concentric central opening of 14 mm. (9/16 in.) in diameter. This disk is pushed down the drawtube to a point about 1·5 cm. (5/8 in.) beyond the lower end of the longest eyepiece used so as not to reduce the field of the latter. With the diaphragm in suitable position, the aspect of the Ramsden's circle should be like 'A' in Fig. 4 and the image will be cleared of all fog or indistinctness caused by stray light.

The Coarse Adjustment

Examine the rack and notice if it has been coated with a heavy layer of grease. A good coarse adjustment should only show traces of lubricant between its contact surfaces and a surplus may be an attempt to cover up mechanical defects.

Try the adjustment from one end to the other of its run; the movement should be smooth and regular, without jerk or backlash. Carefully regulate the compensating screws, with which all stands should be provided, so that the coarse adjustment is not too stiff nor yet loose enough to sink beneath the weight of a triple or quadruple nosepiece with its objectives when the microscope is placed vertically.

Make sure that there is no lateral movement in the coarse adjustment slide. For this, rack the body upwards to about half its total course and then, while holding the limb in the left hand and the body in the right, impart a side to side rocking movement to the whole stand. If any shake or rattle is felt in the coarse adjustment slide, the instrument should be rejected. (*The Microscope* by Drew and Wright.)

Test the delicacy of the coarse adjustment with a 4 mm. (1/6 in.) objective and an eyepiece × 10; it should be possible to focus this objective accurately by means of the coarse adjustment alone. A fraction of a turn of the milled heads should change the focus if there is no wear or slackness in the mechanism.

Observe if the coarse adjustment displaces the body tube exactly along its optical axis. This test is perhaps the most important of all as it can also show if the tube is perpendicular or not to the stage surface. Before attempting this test, great care should be taken that the light cone entering the objective is truly centred with the optical axis as the slightest obliquity in the illumination will completely upset the results obtained. For this, focus the microscope (8 mm. objective, eyepiece × 10) on a clearly visible, but not too large, detail such as a grain of dust on the object-slip. Centre the condenser carefully (see page 78) and then remove the ocular and examine the back-lens of the objective. Incline the mirror this way or that until the back-lens is *uniformly* illuminated; the illuminating cone will then be accurately centred. This can be still further verified by racking the condenser slightly downwards. The image of the light source will then become visible in the objective back-lens and can be brought to its exact centre by means of the mirror.

When the above manipulations have been accomplished, replace the eyepiece and, by means of the coarse adjustment, focus first slightly above and then slightly below the object on the slide. At each change of focus the image of the object should become blurred, *but it should not alter its position.* If the image appears to be displaced say to the right when the objective is above the point of true focus (at 'A' in Fig. 5) and to the left when it is below (at 'B'), that means that the coarse adjustment

is not moving the body-tube along the line of its optical axis 'O—O¹' but along the diagonal 'D—D¹'. The same appear-

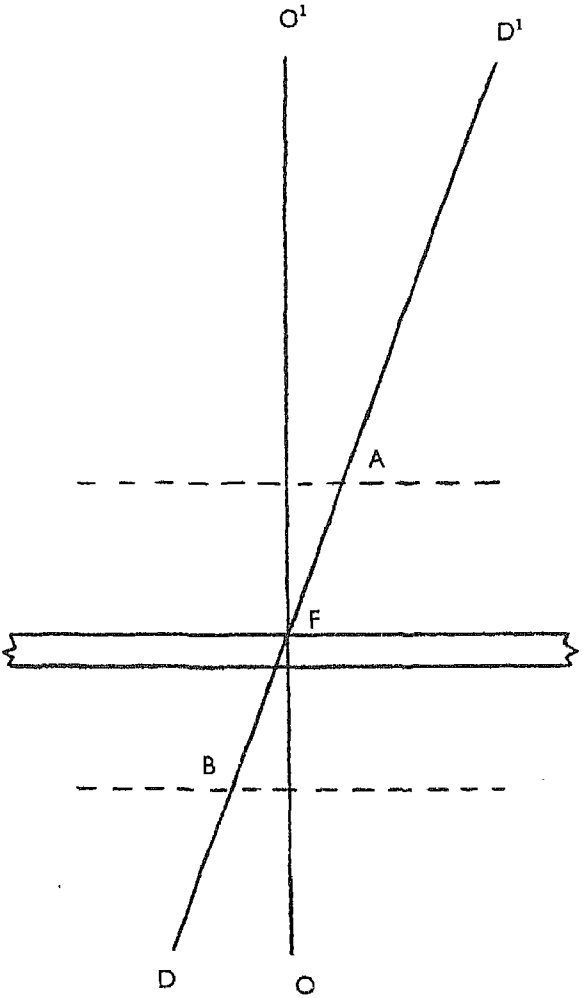

FIG. 5

ances would, of course, be observed if the axis of the body-tube were inclined at an angle equal to 'D¹FO¹' instead of being perpendicular to the plane of the stage. In either case however the microscope should be rejected.

Finally, with a properly proportioned coarse adjustment it should be possible to focus a 40 mm. (1 2/3 in.) objective, or one of even greater focal length, with the latter in place on the nosepiece.

The Fine Adjustment

The sensitivity of the fine adjustment should first be tested with a 2 mm. (1/12 in.) oil- or water-immersion objective. The focus should be obtained surely, rapidly and without the slightest difficulty. No vibration or time-lag should be present in the movement; a fraction of a turn of the milled head should produce an immediate change in the focus at any point of the fine adjustment slide, even when the microscope is inclined to the horizontal.

Make sure that the fine adjustment displaces the body-tube along the line of its optical axis. A 4 mm. (1/6 in.) objective should be employed and the test carried out in the same way as previously described for the coarse adjustment. The same precautions must be taken to ensure accurate axial illumination.

The Stage

The stage should not be less than 9 to 10 cm. (3 1/2—4 in.) wide and its central aperture at least 2·5 cm. (1 in.) in diameter.

In the case of a mechanical stage, whether built-in or detachable, verify under a fairly high magnification (4 mm. objective) if the movements are smooth and regular and the mechanism neither too stiff nor too loose. The slightest turn of the milled heads ought to displace the object-slip without any time-lag or jerkiness.

With the microscope in a horizontal position, make sure that the vertical movement is not so loose as to be displaced by its own weight.

Ascertain if the stage movements take place in a horizontal plane exactly at right angles to the microscope's optical axis. With a 2 mm. (1/12 in.) objective, a flat preparation (a smear of a culture of micrococcus) should remain constantly in focus when different regions of the object-slip are brought into the field by means of the mechanical stage.

Make sure that the centring screws of a circular rotating stage are properly placed and long enough to make the axis of rotation coincide exactly with the optical axis of the microscope.

With a 2 mm. (1/12 in.) objective, see if the stage is of sufficiently rigid construction not to "give" and alter the focus when the stage or substage mechanisms are manipulated.

The Substage

Verify, as with all the other mechanical parts of the microscope, if the substage movements are smooth and accurate, both for the coarse focusing and for the fine adjustment if one is provided.

Make sure, too, that the displacement of the condenser takes place exactly along the optical axis of the microscope. To ascertain this, obtain accurate axial illumination as previously described, place a 4 mm. (1/6 in.) objective on the microscope and close the condenser iris diaphragm almost completely. Remove the eyepiece, observe the objective back-lens and centre the condenser by means of the iris as explained on page 78. Then, with the substage coarse adjustment, move the condenser slowly up and down. The bright disc of the iris will vary in diameter, but it should remain always concentric with the objective back-lens. If this is not the case, the condenser is not being displaced along the optical axis and the instrument should be rejected. While making this test, it can also be discovered if the substage centring screws are well placed and long enough to centre the condenser.

The Mirror

It is much more important than generally thought for the microscope mirror to be of the finest quality as, if it gives multiple images of the light source, the corrections of the condenser are upset and the image suffers in consequence. This defect can be sought for in the following manner: Remove all the optical parts of the microscope, including the condenser. Look vertically down the body-tube and incline the mirror until an image of the light source, preferably a 60 W. opal electric bulb, is seen. Place against this bulb a cardboard or metal screen in which a

slit 10 mm. (7/16 in.) long by 3 mm. (1/8 in.) wide has been cut. Examine the image of this slit in the mirror; if instead of one reflection, bright and sharp along the edges, several reflections are seen, the mirror is defective. This fault may sometimes be remedied by rotating the mirror in its cell until the multiple images are superimposed into a single one. A small scratch is then made on the glass corresponding to another on the metal rim and the mirror is always used in the position thus marked. Multiple images are caused by a faulty parallelism of the upper and lower surfaces of the glass and the great advantage of stainless steel mirrors is that they do not present this defect.

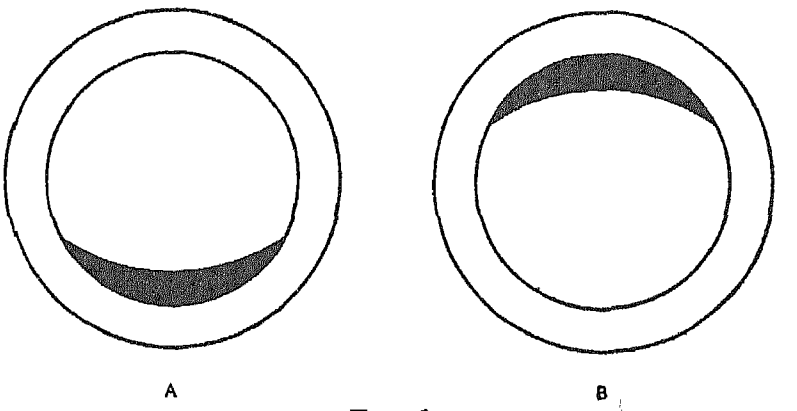

FIG. 6

See if the centre of the mirror coincides with the optical axis of the microscope. This can be ascertained as follows: Centre the condenser with a 4 mm. (1/6 in.) objective (see page 78) and then remove the eyepiece, the objective and the optical portion of the condenser so that the iris diaphragm can be used by itself. Mark the centre of the mirror with a spot of ink, place the mirror in a horizontal position, close the iris almost completely and look down the vertical body-tube. The spot of ink on the mirror should be exactly in the centre of the iris opening.

If the centre of the mirror does not coincide with the microscope's optical axis, and more especially if the mirror is too

small (less than 5 cm. in diameter), much inconvenience may be caused when the microscope is used in an inclined position. To verify this, incline the microscope to about 40 degrees and focus a 4 mm. (1/6 in.) objective on a suitable object after having carefully focused and centred the condenser. The most suitable light source is a 60 W. opal bulb. Open the iris diaphragm to its fullest extent, remove the eyepiece and examine the objective back-lens. If the mirror's centre is badly placed, one of the two aspects of Fig. 6 will be seen. The dark arc represents the image of the *metal rim* of the mirror. If the arc in question is towards the bottom margin of the back-lens as in 'A', the arm supporting the mirror is too short; it is, on the contrary, too long if the appearance is as in 'B'.

The only test for the concave mirror is to make sure that its radius of curvature is such that an image of the light source can be projected in the plane of the object on the stage. Remove all the optical parts of the microscope and set up in front of the light source (60 W. opal bulb) a cardboard screen in which a slit about 8 mm. (1/3 in.) long by 3 mm. (1/8 in.) wide has been cut. Place on the stage a ground-glass slide, with the ground surface downwards, or even a piece of ordinary tracing-paper. It should be possible to focus an image of the slit on to the ground-glass.

In all tests the light source is placed at a distance of 250 mm. (10 in.) from the mirror.

2. TESTS FOR THE OPTICAL SYSTEM

Objectives

It is very difficult to pass judgment on an objective without having a long experience of microscopy. The novice had better buy only new objectives and rely on firms with an established reputation. It might interest him however to try his hand on the following test objects which, with a little practice, will also enable him to gain some insight into the optical value of any objective which might pass through his hands.

For low powers up to objectives of 8 mm. (1/3 in.) F.L., an excellent test object is a blow-fly's tongue mounted in Canada

balsam. The numerous hairs around the tongue's margin should be sharp and clear-cut, ending in finely tapered points without any fuzziness or reduplication. As hairs of all sizes are present, those suitable for the power of the objective used can easily be brought into the field.

For high-power dry objectives, from 6 mm. (1/4 in.) upwards, a dry strewn-slide of the diatoms *Pleurosigma angulatum* or *Nitzschia scalaris* is excellent. The frustules should appear perfectly flat and covered with minute well-defined dots which change from black to white according to the focus. This test may be a shade too difficult for the 6 mm. (unless it is an apochromate), but it is well within the range of a 4 mm. objective.

A good test for water- and oil-immersions is a slide of *Mycobacterium tuberculosis* dyed red by the Ziehl-Neelsen method and counterstained with methylene blue. The bacilli should be seen clearly as little pinkish-red rods against the pale blue background and their characteristic 'beaded' appearance should be distinctly revealed.

All the above tests are for axial illumination with as large a cone as the objective will stand.

Eyepieces

The only practical test applicable to eyepieces is to find out if the diaphragm between the two lenses is in the right position. To do this, look through the ocular towards a white, well-lit surface. The margins of the field should appear quite sharp and clear-cut. If this is not the case, it can be remedied by displacing the diaphragm up or down until a satisfactory result is obtained.

Condensers

Find out with a pair of callipers if the iris diaphragm is concentric with the optical axis of the condenser and if this axis is in the centre of the iris opening when the latter is closed to its utmost. A spot of ink in the centre of the back-lens of the condenser will be of assistance in this test.

The degree of chromatic correction of an achromatic condenser may be judged by projecting an image of the light source in the plane of a suitable object on the stage and examining the

image in question with a 25 mm. (1 in.) objective—if possible an apochromate. The margins of the image should appear bright and sharp against an almost black background without showing any traces of colour fringes.

The definition and spherical correction of some achromatic condensers may be tested, if their mount is small enough to be provided with a R.M.S. objective screw, by placing them on the microscope and using them as ordinary objectives. Achromatic condensers are almost as carefully corrected as objectives and the image given by them should be nearly as good. It should not be forgotten in this test that condensers are corrected to work through the thickness of the *slide*; consequently the latter must be placed upside down on the stage with the cover-glass underneath.

Should it be thought necessary to check the numerical aperture of a condenser, this can be done by comparing it with an objective of equal or approximately equal numerical aperture. In the case of an achromatic immersion condenser of 1·30 N.A. for example, proceed as follows:

Immerse the condenser with cedar-wood oil, centre and focus it carefully on a suitable object mounted in Canada balsam. For these preliminary adjustments a 25 mm. (1 in.) objective should be used as described on page 76, the light source being a 60 W. opal bulb at a distance of 250 mm. (10 in.). Then focus the object with a 2 mm. (1/12 in.) oil-immersion objective of 1·30 N.A. and shift the slide a little so as to bring an empty portion of the preparation into the field. Open the condenser iris to its widest, remove the eyepiece and examine the objective back-lens. The whole of the latter should be uniformly filled with light. With the same test, an achromatic dry condenser should entirely fill with light the back-lens of a 0·90 or 0·95 N.A. objective.

Finally, when selecting an immersion condenser, make sure that its focus is neither too short nor too long. In the first case it will be impossible to focus it through a moderately thick slide, and, in the second, the oil film will be continually breaking contact between the slide and the condenser front-lens. The focus should be about 1·6 mm. (1/15 in.) for a 1·30 N.A. condenser, and 1·3 mm. (1/16 in.) for one of 1·40 N.A.

No account has been taken in this chapter of scratched, cracked or otherwise *damaged* optical parts, but this contingency should not be overlooked. A pocket magnifier for examining the back- and front-lens of eyepieces, objectives and condensers is invaluable for tracking down defects and blemishes, including the all too frequently forgotten film of immersion oil.

PART TWO

TECHNIQUE OF MICROSCOPICAL OBSERVATION

CHAPTER IV

ILLUMINATION BY TRANSMITTED LIGHT

There are three methods of illuminating an object that has to be examined under the microscope:

The first consists of sending the light directly into the objective through a transparent or semi-transparent object. This is *transmitted light illumination* in which the object is seen on a bright field.

In the second method, the light is directed very obliquely on to the object so that the direct rays cannot enter the objective front-lens, but only the rays which are refracted and deviated from their path by the object under examination. The latter then appears to be shining by its own light against a black background and the method is known as *diffracted light* or *dark-ground illumination.* It is mostly used for very minute objects or particles floating in a liquid medium.

The third method, generally reserved for opaque objects of some size, consists of illuminating them either from above or from the side so that they are seen by the light they reflect. This is *illumination by reflected light.*

Transmitted light illumination may be: (1) Axial. (2) Oblique.

In *axial illumination* the axis of the illuminating cone which enters the objective is co-axial with the latter's optical axis. This is the outstanding method for microscopical work, the one which gives the sharpest and clearest images and those which are the nearest to reality.

In *oblique illumination* the axis of the illuminating cone entering the objective forms a certain angle with the latter's optical axis. The term also includes *annular illumination* in which the central rays of an axial cone are cut off by a round concentric stop which is not however large enough to produce dark-ground illumination.

1. AXIAL ILLUMINATION

The technique for axial illumination differs slightly according to whether low, medium or high powers are used, but it is always based on four fundamental rules as formulated by Instructor-Commander M. A. Ainslie in the 'Journal of the Photomicrographic Society', Vol. IX, No. 2, June 1920.

(a) The circular shape of the objective lenses must not be impaired.

(b) The object to be examined must be lit by a solid cone of illumination the axis of which coincides with the optical axis of the microscope. When viewed from above, the back-lens of the objective should show a round circle of light in its centre, a result which can only be obtained if all the optical parts of the microscope are accurately centred.

(c) This circle of light must be as large as possible compared with the back-lens. Its diameter should not be less than 2/3 of that of the back-lens; if possible it should be 3/4 or even more. In other words the maximum possible of the numerical aperture of the objective must be utilized by employing the largest illuminating cone that it can stand.

(d) The circle of light must present a surface of uniform brightness.

To these four rules another two can be added which derive from the previous ones:

(e) Use a suitable light source.

(f) Neutralize the errors introduced into the objective's corrections by various causes, one of the principal of which is the influence of cover-glass thickness.

The technique of microscopical observation will now be described, beginning with low-power objectives which are the simplest and easiest to use.

To satisfy the four fundamental rules already enumerated, a certain number of operations must be carried out in a sequence which will always remain the same whatever the objective used. Some people will perhaps think these manipulations tedious and unnecessarily meticulous. It is therefore advisable to stress from the outset that if any of these details are neglected, it will be impossible to get the *maximum* of resolving power from an

objective. With practice too, as eye and hand become more expert, the various adjustments are made smoothly and automatically until finally they can be carried out in far less time than it takes to describe them.

Technique to be employed with very Low-Power Dry Objectives

APPARATUS USED

Light Source

One of the best light sources, for visual observation at least, which brings out to the full the optical qualities of objectives of large numerical aperture, is the old-fashioned oil lamp as made for the microscope by most opticians. This burns a 1·5 cm. (5/8 in.) flat wick and has an all metal chimney which lets the light out through a rectangular opening taking ordinarily 76 x 26 mm. (3 x 1 in.) glass slips or slightly wider. For low powers, the flat of the flame is turned towards the mirror; the edge is used with medium- and high-power objectives.

A 60 W. opal electric bulb also gives excellent results, especially if a metal screen with a circular hole 2 cm. (3/4 in.) in diameter is placed in front of the bulb and in contact with the latter.

With both these illuminants we have been able to resolve a Realgar mounted *Amphipleura pellucida* (2 mm. apochramatic oil-immersion objective of 1·30 N.A., achromatic and aplanatic immersion condenser of 1·30 N.A.) using a 3/4 *axial* cone. This shows that they are both suitable for delicate visual work, though the oil lamp was distinctly the more satisfactory of the two. The author has heard much good of the *ribbon filament* electric lamp for visual work, but he has not, so far, had the opportunity of thoroughly testing one.

Incidentally, it may be mentioned that two of the *worst* illuminants for microscopy—which are still often used—are the naked filament and the 'ground-glass' (*not* opal) electric bulbs. With both these it is impossible to obtain 'critical illumination' as described later (page 85), as the image of the filament or the

grain of the ground glass superimposes itself on the image of the object examined.

Ordinary daylight, especially when reflected from a white wall or cloud, gives fairly good results with *low* powers, but it is too uncertain and quite unsuitable for delicate work.

In the present chapter an oil lamp with a 1·5 cm. wick will be taken as an illustration. It should be noted however that the technique remains the same in its essential details for all light sources.

Microscope Stand

Medium model, as described in Chapter III.

Optical System

Objective: A 50 mm. (2 in.) achromatic objective, or one of lower power.

Eyepiece: A Huyghenian ocular of 50 mm. F.L. × 5.

Condenser: None.

With very low magnifications, it is often difficult (unless a special low-power condenser of very long focal length is used) to fill the whole field, or even a fair portion of it, with light. This difficulty can sometimes be overcome by removing the front-lens of a medium-power condenser, but with objectives of 25 mm. (1 in.) F.L. and longer it may be more convenient to use the *concave* mirror alone without any other illuminator. In this case the optical part of the condenser should be removed and the iris diaphragm in the condenser mount can then be used by itself for regulating the light. Excellent results are obtained with certain objects by utilizing, in place of the mirror, a disk of glazed cardboard or even of white paper.

Mirror

The concave mirror.

Slide to be Examined

Any suitable object mounted dry or in some medium; e.g. a fish scale, mounted dry.

Cover-glass Thickness

Theoretically as near as possible to that for which the objective is corrected. In practice this is of little or no importance with very low-power objectives, from 16 mm. (2/3 in.) F.L. and longer.

PRELIMINARY MANIPULATIONS

1. Set up the microscope and incline it at a comfortable angle—unless the stage has to be kept horizontal owing to the fluidity of the slide to be examined.

2. Set the drawtube for the mechanical tube-length for which the objective used has been corrected.

Incidentally, one should verify once and for all whether the figures on the drawtube scale include the length of the revolving nosepiece or not.

3. Screw the objective on to the microscope and place the eyepiece in position.

If the run of the coarse adjustment slide is too short to allow the focusing of a very low-power objective, screw the latter into the R.M.S. thread at the lower end of the drawtube. The mechanical tube-length becomes much shorter, but this is of minor importance with objectives of 16 mm. (2/3 in.) F.L. and more as these are very insensitive to variations of tube-length as well as of cover-glass thickness.

4. Place the lamp at a distance of 250 mm. (10 in.) from the mirror.

This does not matter when no condenser is used, but it is of distinct importance with achromatic condensers which are generally corrected for this distance. In fact the light source should be placed at 250 mm. from the *back-lens of the condenser* and consequently the distance between the latter and the mirror should be subtracted from the total distance to the illuminant.

5. Adjust the height of the light source so that the flame (or bulb) is approximately on the same level as the mirror.

6. If an oil lamp is used, turn the *flat* of the flame towards the microscope.

7. Place the object-slip on the stage.

Much trouble will be saved in the long run if a work board

of some form can be made with shallow recesses to receive the microscope foot, the lamp and any other accessories which may be frequently employed. In this way, the correct positions need only be measured once, after which the whole outfit can be set up again at any time with the minimum trouble and delay.

8. Incline the mirror until the light from the lamp is directed up the body-tube.

Beginners sometimes meet with some difficulty in achieving this, though this is hardly likely to happen with very low powers. If, however, it should be the case, the obstacle can be overcome by removing both the objective and the eyepiece and looking down the empty tube on to the mirror. The right position for the latter can then be easily found.

FINAL MANIPULATIONS

1. Focusing the Objective

Look at the stage from the side and bring the object to be examined as vertically as possible beneath the objective. Rack the latter downwards by means of the coarse adjustment milled heads until it nearly touches the object. Then, with the eye at the ocular, rack the body very gently upwards until the object comes into view. It can then be finally and accurately focused. With low powers, up to 16 mm. (2/3 in.) F.L. and even less, the coarse adjustment only should be used for the final as well as the preliminary focusing, both to save time and to prevent unnecessary wear and tear to the fine adjustment.

Sometimes the beginner fails to see the object when racking up the body-tube owing to the former not being exactly beneath the objective. When this happens, it is best to lower the objective again and to rack it up very slowly until dust specks or other defects of the object-slip appear in the field of view. When this has been achieved, a slight displacement of the slip to the one side or the other should bring the object into the field. It must not be forgotten that the microscope reverses the image and that consequently a displacement of the slip from right to left will move the object in the field from left to right and vice versa.

If, in spite of all attempts, the beginner still fails to locate the

object, two further methods may be tried: (a) If the object is fairly large, remove both eyepiece and objective, look down the empty tube and bring the object as near to the centre as possible. It will then, in all probability, be in the field when the optical parts are replaced. (b) Remove the eyepiece and examine the objective back-lens. The object will generally be seen (or will appear after a few displacements of the slide) at some part of the back-lens' periphery. From there it can be brought into the centre and should be visible in the field after replacing the eyepiece. This last method is more suitable than the first for small objects as they appear somewhat magnified when seen in the back-lens. It can be used even with high-power objectives.

2. Focusing the Mirror

Move the mirror up or down upon its tail-piece until an image of the lamp flame comes into view in the field superimposed upon that of the object under examination. Look at the margins of this flame image and adjust the mirror until they appear as sharply defined as possible, then tilt the mirror until the flame is centred in the field. If the image of the flame is too broad for both margins to be seen at once, the focusing can be done on one margin only by decentring the flame slightly. If the illuminant is an opal bulb, the periphery of the 2 cm. (3/4 in.) opening of the metal screen placed in front of it can be used for focusing, or a small cross can be inked on the glass and wiped off afterwards.

If the illuminated portion of the field is too small, an oil lamp with a broader wick can be used or the metal screen in front of the opal bulb removed. It should be kept in mind however that, for visual work at least, it is not always necessary or even advisable to have the whole field filled with light. Definition is often better and eye strain less if only the central area is illuminated.

It is often advantageous, instead of the concave mirror alone, to employ one of the special low-power substage condensers. With these the *plane* and not the concave mirror should be used, and the technique is similar to that described for the achromatic condenser a little further on.

3. Regulating the Illuminating Cone

Most beginners have a tendency to close the substage iris far too much in attempting to reduce glare and to increase contrast. This is a dangerous proceeding however as many of the finer details are lost and the whole image is blurred by diffraction fringes and other spurious effects. The best way of regulating the illuminating cone is, once more, to remove the eyepiece and examine the back-lens of the objective. The iris is slowly closed until a portion of the outer zone of the back-lens equal to a quarter of its total surface has been obscured. The eyepiece is then replaced and the last slight readjustments are made according to the aspect of the object as seen in the field. These manipulations assume a greater importance with medium and high powers and are dealt with in a more detailed manner on page 84.

4. Regulating the Intensity of the Light

Here again the novice will often fall back on the iris diaphragm if he finds the microscope field too brightly illuminated, with the unsatisfactory results previously mentioned. It is far better to reduce the intensity of the illuminant if the latter is too bright (as is generally the case in visual work) by means of light filters placed either in front of the lamp or in the stop carrier of the substage condenser. This subject is treated at greater length on page 91.

Technique to be employed with Medium- or High-Power Dry Objectives and an Achromatic and Aplanatic Dry Condenser

APPARATUS USED

Light Source

Oil lamp or 60 W. opal electric bulb as described previously.

Microscope Stand

Medium or large sized stand as described in Chapter III.

Optical System

Objective: A 4 mm. (1/6 in.) F.L. achromatic or apochromatic objective of 0·70 to 0·95 N.A.

Eyepiece: A 25 mm. F.L. Huyghenian or compensating ocular × 10.

Condenser: An achromatic and aplanatic dry condenser of 0·90 to 0·95 N.A.

As the numerical aperture of a dry objective cannot theoretically surpass 1·0 and in practice is never higher than 0·95 even for apochromates, it is evident that a dry achromatic and aplanatic condenser can provide it with the largest light cone that it is capable of utilizing. One can however, if one prefers it, use an immersion condenser united to the object-slip by a drop of cedar-wood oil so as to avoid the necessity of having to correct the condenser for different slide thicknesses. It is owing to this last quality that the immersion condenser is particularly valuable in photomicrography, more especially as it does away with reflections on the under side of the glass slide and thus allows more light to reach the objective.

One can also—to avoid having two condensers—use the immersion condenser dry. The small loss of definition, due to the fact that the condenser has been corrected for immersion, is not sufficient, on the whole, to be really appreciable with dry objectives. A much more important factor in obtaining the best results is never to omit the correction for the thickness of the slide when a dry achromatic condenser is used. This correction has an influence not only on the sharpness of the image, but also on its brightness and can do much to improve contrast in photomicrography.

If a special low-power or an Abbe condenser is employed, the technique is similar to that for the achromatic condenser except that the correction for object-slip thickness need not be so thorough. In fact this correction would be impossible to effect with an Abbe condenser owing to the latter's enormous aberrations and need not even be attempted.

Mirror

The *plane* mirror, preferably one of stainless steel. (The plane mirror is always used in conjunction with a condenser so as not to upset the latter's corrections.)

Slide to be Examined

Any suitable object mounted dry or in some medium. E.g. A slide of the diatom *Pleurosigma angulatum*, mounted dry. (With this object six diffraction spectra will be noticed when the objective back-lens is examined. These do not affect the manipulations described in any way and can be disregarded.)

Cover-glass Thickness

Theoretically as near as possible to that for which the objective has been corrected. In practice, however, this is not of great importance as the aberrations introduced by variations of thickness can be neutralized, as explained on page 85.

Slide Thickness

As near as possible to that for which the condenser has been corrected. Small variations can be neutralized as described on page 79, but on no account should the thickness exceed the length of the condenser's focus. Most dry condensers are corrected for a slide thickness of 1·5 mm. and will not focus through one of much greater thickness.

PRELIMINARY MANIPULATIONS

1. Set up the microscope and incline it at a comfortable angle.
2. Set the drawtube for the mechanical tube-length for which the objective has been corrected.
3. Screw the 4 mm. (1/6 in.) objective and also one of 25 mm. (1 in.) F.L. on to the revolving nosepiece. Place the eyepiece in position. The weaker objective will be used for all the *preparatory* operations and should be revolved into the optical axis of the microscope.
4. Place the illuminant at a distance of 250 mm. (10 in.) from the lower lens of the condenser (see page 71).
5. Adjust the height of the illuminant so that the flame (or bulb) is approximately at the same height as the mirror.
6. If an oil lamp is used, turn the *edge* of the flame towards the mirror.
7. Place the object-slip on the stage.

8. Incline the mirror until the light from the lamp is directed up the body tube.

FINAL MANIPULATIONS

With medium and high powers, the final manipulations are divided into two operations: (A) The preparatory focusing and centring of the optical system. (B) The final focusing and centring of the optical system.

A. PREPARATORY FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

These preparatory operations are carried out with the 25 mm. (1 in.) F.L. objective.

1. Focusing the Objective

The 25 mm. (1 in.) F.L. objective is focused on the object-slip as described previously for low powers.

The part of the object which is to be examined is brought carefully to the centre of the field so as to make it easier to locate when the 25 mm. (1 in.) is replaced by the 4 mm. (1/6 in.) F.L. objective.

2. Focusing the Condenser

Raise or lower the condenser very slowly by means of the sub-stage focusing mechanism until a sharp image of the lamp flame is seen superimposed on the sharply focused object. The angle of the mirror is then adjusted until the flame image is brought into the centre of the field.

When the illuminant is an opal electric bulb, it may be difficult to focus its central region if its extensive uniformly bright surface fills the whole microscope field. In that case the mirror can be tilted slightly until the margin of the bulb appears. After this has been focused, the image is again centred for the field to be evenly illuminated. If a screen with a circular opening has been placed in front of the bulb, its edges can be used for focusing, or else a mark can be inked on the glass and afterwards wiped off.

It will occasionally be found that it is impossible to focus the image of the illuminant as the front-lens of the condenser comes into contact with the under surface of the object-slip. This means that the slide is too thick for the condenser to be able to focus through it. There is unfortunately no remedy for this state of affairs. If the difference is very small, the focus of the condenser may sometimes be lengthened sufficiently by bringing the lamp nearer to the mirror. This has the drawback however of reducing the numerical aperture of the condenser and upsetting the accuracy of its corrections.

3. Centring the Condenser

The condenser iris is almost completely closed, the eyepiece is removed and the objective back-lens is examined. The latter will present the aspect of a dark circle with a tiny bright disk somewhere near its centre. This disk is the iris opening and, by means of the centring screws, it should be brought exactly into the centre of the back-lens. It is sometimes rather difficult to judge if the iris opening has been accurately centred. This can be checked by opening the iris slightly and seeing if its periphery remains concentric with the outer zone of the back-lens. The condenser has now been centred with the optical axis of the *25 mm. objective.* As will be seen presently, it will be necessary to modify this centring slightly when changing to the 4 mm. objective.

If, when the eyepiece has been replaced, it is seen that the image of the illuminant is no longer in the exact centre of the field, it is brought there by a slight change in the angle of the mirror. The centring screws should on no account be touched.

Instead of removing the eyepiece each time one wishes to examine the objective back-lens and thus allowing dust to enter the microscope tube, it is preferable to enlarge the Ramsden's circle by means of a magnifying glass of short focal length magnifying about 10 diameters. An ordinary Huyghenian ocular, × 10, will serve if held upside down over the microscope eyepiece and near enough for their eye-lenses to be almost touching.

It might be worth while mentioning here that, in stands which have no centring screws to the substage, the condenser can often

be approximately centred by turning it this way or that in its plain tubular underfitting. It is rare for this tube to be a perfect cylinder and a position of optimum centring can generally be found.

The method of centring the condenser described in the above paragraphs is the best and quickest, but it may present some difficulty with certain types of condenser. With the Abbe, for instance, the enormous aberrations make the image of the iris rather indistinct when viewed in the objective back-lens.

In such a contingency, two other methods may be tried:

(a) A small spot is made with ink or a fine pointed chinagraph pencil in the centre of the upper surface of the condenser front-lens. This mark is then viewed through the microscope as if it were an object and brought to the centre of the field by means of the centring screws. The spot is afterwards wiped off. As can be seen, this method is a clumsy one as the condenser has to be marked anew each time the objective is changed if accuracy is desired. It also entails removing the object-slip or throwing the condenser out of the optical axis and it would, of course, be impracticable with immersion objectives. If the spot is made *very small*, it need not be wiped off when only low-power objectives are used as the deterioration of the image would be imperceptible.

(b) The iris diaphragm is almost completely closed and its aerial image is focused with a low-power objective. To achieve this, the objective and the condenser are first focused in the ordinary way upon an object-slip. The latter is then removed from the stage or moved aside, the iris is closed and the objective slowly racked upwards until the aerial image is seen. This image is formed about 3 mm. (1/8 in.) above the surface of the condenser front-lens and, when found, should be brought to the centre of the field by means of the substage centring screws. Here again, this method is very difficult to use with medium- and impossible with high-power objectives.

4. Correcting the Condenser for Slide Thickness

Most dry achromatic condensers are corrected to work through a slide 1·5 mm. (1/16 in.) thick. This includes, of course,

the layer of mounting medium between the slide and the object if the latter is not in intimate contact with the glass. To obtain the best results, the condenser should accordingly be corrected for any departure from the right slide thickness. The procedure is as follows:

The objective and the condenser having already been focused on the object as previously described, slowly raise and lower the objective by means of the coarse adjustment. Whilst doing this, examine the image of the lamp flame (or the edges of the round opening in the metal screen if an opal bulb is the illuminant) on the object-slip. If this image presents the same aspect when the objective is moved a similar distance on either side of its correct focus, then the slide thickness is the right one. If, when the objective is raised, the flame image at once becomes hazy with ill-defined edges, while, when the objective is lowered, the image remains sharp for a longer time, it means that the condenser is *under-corrected* owing to the slide being *too thin*. If the appearances are reversed, the condenser is *over-corrected* by *too thick* a slide. Both these defects can, in some cases, be remedied as described below:

(a) Condenser under-corrected by too thin a slide. The thickness of the slide can be increased or 'built up' by fixing one or more cover-glasses to its under surface with a little Canada balsam or cedar-wood oil.

If the difference is not too great, it can be neutralized by slightly unscrewing the front lens of the condenser.

After using either of the above methods, test the correction by raising and lowering the objective as described previously.

(b) Condenser over-corrected by too thick a slide. Unfortunately there is no real remedy for this condition. If the difference is very small, it can sometimes be corrected by bringing the lamp nearer to the mirror. This method however is not to be recommended, except if absolutely necessary, as the light source should remain at the constant distance of 250 mm. (10 in.) from the back-lens of the condenser if the best optical performance is to be obtained.

Correcting the condenser for slide thickness is not as important as correcting the objective for cover-glass thickness (see page

85), but it should not be neglected. It not only has a perceptible influence on the quality of the microscope image, but it also considerably shortens exposures in photomicrography. According to Dr. A. C. Coles (*Critical Microscopy*, p. 60), a too thin object-slip sometimes requires double the exposure needed for one of the correct thickness under a magnification of 1,000 diameters.

The above technique can be used with all dry condensers, including those of long focal length for low-power objectives.

Refocus both objective and condenser after the manipulations just described have been concluded.

B. FINAL FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

The 25 mm. (1 in.) objective, used for the preparatory operations, is now replaced by the 4 mm. (1/6 in.) objective.

Before turning the revolving nosepiece, especially when working with high-power objectives having a short working distance, one should be careful to rack the body-tube slightly upwards for fear that the longest objective might strike the object-slip. This is an accident that can easily happen unless all the objectives have been carefully parfocalled. It is perhaps worth while pointing out here that a revolving nosepiece should always be rotated in one direction only. The right direction, generally clockwise, is often indicated by an engraved arrow; it should be adhered to if accuracy of centring is to be retained and unnecessary wear avoided.

When changing object-slips, it is also prudent to rack a high-power objective slightly upwards or the front-lens may be scratched if the second slide is appreciably thicker than the first.

1. Focusing the Objective

The focusing of the 4 mm. (1/6 in.) objective is carried out in the same manner as described for the 25 mm. (1 in.) on page 72. It requires however more care, especially when the objective is being racked down, as, owing to its much shorter working distance, there is more danger of the front-lens getting damaged

by being forced down on the object-slip. When racking up, the eye should watch carefully for the moment when the object first appears in the field, and the final exact focusing can then be finished by means of the fine adjustment.

If the object has been previously brought into the centre of the field of the 25 mm. objective, there is generally not much delay in relocating it with the 4 mm., but, should any difficulty be experienced, the object can usually be found by examining the objective back-lens as explained on page 96.

It must not be forgotten, of course, that it will be *impossible* to focus an object if the thickness of the medium in which it is mounted added to that of the cover-glass is too great. The longest working distance for a 4 mm. objective is only about *1 mm. at the most.*

2. Focusing the Condenser

The whole point of focusing the condenser is to fill the back-lens of the objective with as much light as possible and thus utilize the maximum of its numerical aperture. A condenser which does not focus an image of the source of light accurately in the plane of the object to be examined, cannot illuminate the whole of the back-lens of a high-power objective. This can be easily proved by experiment. Few, if any, illuminants however consist of a single plane and a little latitude in the focusing is consequently permissible. In the case of the oil lamp for instance, either the anterior, the middle or the posterior plane of the flame may be focused. One need only utilize the plane which illuminates the back-lens to the best advantage. To achieve this, the condenser iris is widely opened, the eyepiece is removed and the objective back-lens is examined. As the condenser has already been brought very near to its final focus with the 25 mm. (1 in.) objective, the aspect of the back-lens will resemble that represented in 'A' of Fig. 7 where an enlarged image of the lamp flame is seen bordered on either side by a dark arc. (The various aspects of the objective back-lens are more distinct if the object-slip is slightly displaced so as to bring a fairly empty region of the slide into the microscope field.) If the condenser is then slowly raised—by means of the fine adjustment, if the substage

is provided with one—the image of the flame becomes wider, but, at a certain moment, instead of an evenly bright surface, two dark elongated spots suddenly make their appearance as in 'C'. The last position of the condenser before these spots are seen, when the back-lens is uniformly illuminated as in 'B', is the focus at which the objective will work at its best.

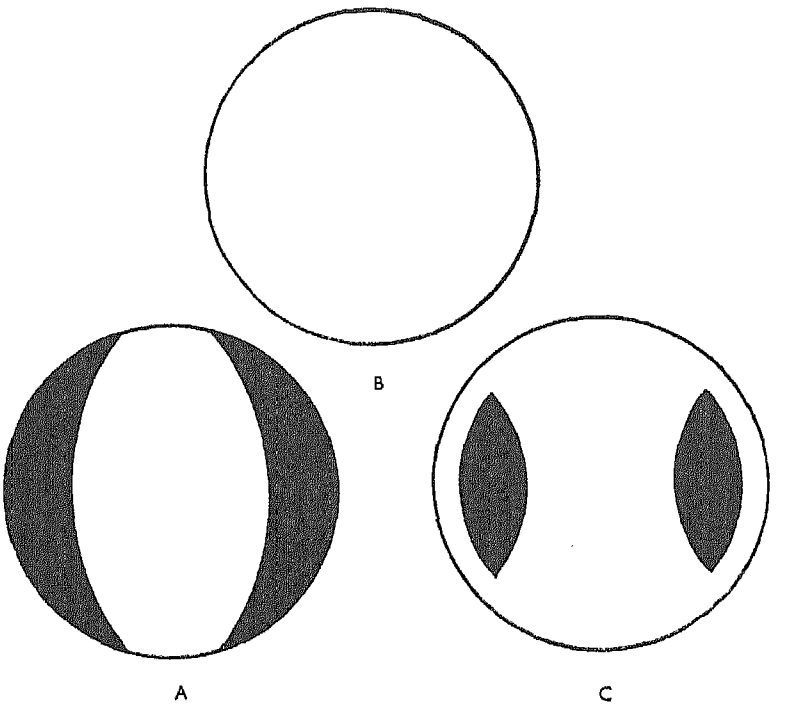

FIG. 7

It will sometimes be noticed, especially with low-power eye-pieces, that the edges of the field are less brightly illuminated than the centre owing to the elongated shape of the lamp flame. This is of no importance in visual work, the centre of the field being mostly utilized, and one should be careful not to alter the position of the condenser as this may cause a deterioration of the objective's optical performance. For photomicrography, where a uniformly lit field is a prime consideration, other

methods, including the use of stand condensers,[1] are described on page 144.

For lower powers than the 4 mm. (1/6 in.) objective, it may sometimes be advisable to turn the flat of the flame towards the mirror to illuminate a larger surface of the field. For the same reason, the front-lens of the condenser may be removed when working with objectives of a greater focal length than 8 mm. (1/3 in.), or a special low-power condenser may be used.

3. Centring the Condenser

The condenser has already been centred with the 25 mm. (1 in.) objective, but, as the optical axes of two objectives almost

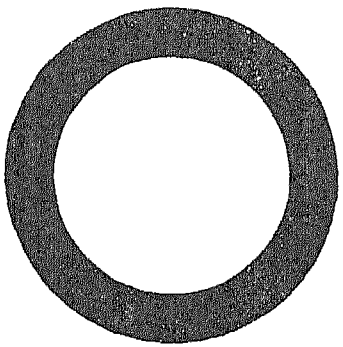

FIG. 8

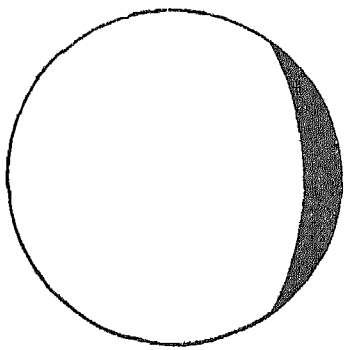

FIG. 9

never coincide, it will have to be recentred for the 4 mm. (1/6 in.) objective. This is done by examining the objective back-lens exactly as described for the 25 mm. objective. After the final centring of the condenser, it may happen (when the iris has again been opened to its full) that the back-lens of the objective will show a dark arc on one side or the other, as in Fig. 8, instead of being uniformly filled with light as before. To remedy this, the angle of the *mirror* should be slightly altered until the back-lens is once more filled with light.

4. Regulating the Illuminating Cone

As already mentioned for low powers, the amount of light

[1] Also called 'bull's-eye condensers'.

that should be allowed to enter the objective is best regulated by observing the back-lens. The great majority of objectives cannot stand an illuminating cone of more than 3/4 of their numerical aperture (see Fig. 8). The condenser iris should accordingly be closed until a peripheral zone of the back-lens of the objective corresponding to 1/4 of its total surface has been blocked out. Only long and patient trials can show whether a given objective belongs to the very few exceptions that can stand a larger cone.

The last delicate adjustment should be made, with the eye at the ocular, from the aspect of the microscope image. Certain objects, such as heavily stained ones, can stand slightly larger cones than others. Consequently the iris should be opened or closed *very slightly* until the best effect is obtained. One should however be careful not to depart too far from the 3/4 cone and, above all, *not to close the diaphragm too much.* Closing the diaphragm increases the contrast of some of the details, but at the expense of the optical value of the image. Not only do the more delicate structures disappear entirely, but diffraction effects are produced which alter the aspect of the remaining details and often create appearances which have little relation to reality.

The method of illumination just described, where the object is at the apex of a solid light cone which uniformly illuminates at least 3/4 of the objective back-lens, is known as *critical illumination*, a term first proposed by Mr. E. M. Nelson.

The image given under critical illumination by a good quality objective carefully corrected for cover-glass thickness (see next paragraph) is called a *critical image*. The goal of all microscopical technique is to obtain this critical image.

5. Correcting the Optical System for Spherical Aberration

(a) The Objective

With dry and water-immersion objectives, there exists between the cover-glass and the objective front-lens a medium with a refractive index very different from that of glass (air = 1·0, water = 1·33, glass = 1·54). The system does not consequently form a homogeneous whole and the corrections of the

objective for spherical aberration are upset unless a cover-glass of exactly the right thickness is employed. It is easy to see however that it is impossible for this condition to be often fulfilled as the thickness of the mounting medium must also be taken into consideration and even that of the object itself. In thick specimens, as will be seen further on, the correction must be altered each time different planes of the object are examined.

Correction for cover-glass thickness is extremely important when working with dry or water-immersion objectives because, if it has not been carefully carried out, it is impossible to obtain the best definition and to see the more delicate details. In extreme cases, the whole object seems to be dimmed by a milky haze. With a little practice, the eye soon recognizes the appearance given by a well-corrected objective and one that is not strikes it as disagreeably as a badly focused image. In fact it *is* a badly focused image.

All high-power water-immersions and some high-power dry apochromates are provided with a correction collar to correct for cover-glass thickness. This accessory is almost never seen on dry achromatic and semi-apochromatic objectives and in these the necessary adjustments must be carried out by means of the drawtube.

Before explaining the actual technique, we shall describe the various appearances on which the adjustments for cover-glass corrections are based.

In Fig. 10, 'A' represents an objective working through a cover-glass of the right thickness for which it has been corrected. Its central and peripheral zones are all in focus simultaneously on the object. If the objective is moved upwards and downwards so as to displace its focus an equal distance on either side of the object and bring into the plane of the latter first the plane 'c' and then the plane 'b', the same appearances will be observed in both cases. For both displacements of the focus, the image of the object (supposed punctiform) will expand into a more or less blurred disk with a darker outer zone.

In 'B' of the same figure the effects of a too thin cover-glass are seen. The spherical correction is upset, the objective has become under-corrected and, when its central regions are in

focus on the object, its marginal zones come to a focus nearer to the front-lens. If the objective is racked upwards to make the plane 'c' coincide with the plane of the object 'a', the image expands into a nebulous disk which is *darkest in the centre*. If, on the other hand, the objective is racked downwards to bring the plane 'b' on a level with 'a', the object will have passed through the foci of the various marginal zones. Its image will assume the aspect of a *sharp dark ring* which becomes larger and hazier as the front-lens approaches the object.

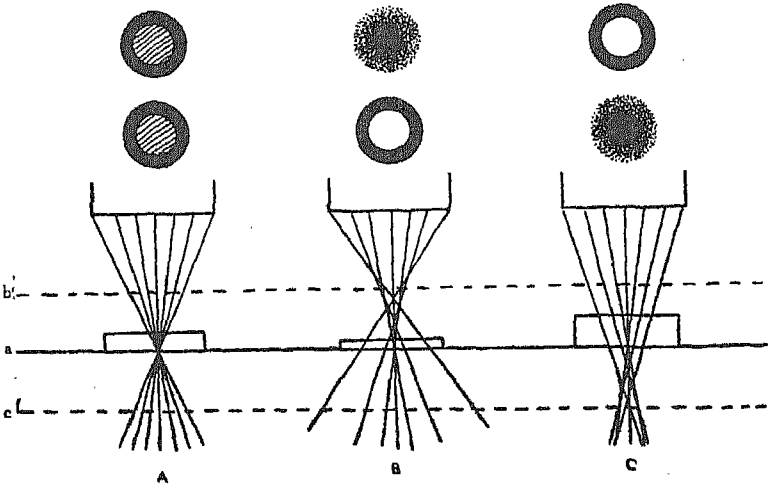

FIG. 10

'C' shows an objective over-corrected by too thick a cover-glass; when its central zones are focused on the object, the foci of its marginal zones are too distant. If the objective is racked up or down, the appearances observed are just the contrary to what they were with an under-corrected objective. A nebulous disk with a darker centre appears when the objective is lowered and a sharp dark ring when it is raised.

When a bright point of light on a dark background is examined in the same way (as when a dark-ground illuminator is used) the appearances are identical, except that in place of a dark disk a bright disk will be seen and a bright instead of a dark ring.

To sum up, all the various appearances can be condensed into the following table:

Well-corrected Objective. Same appearances on either side of the true focus, i.e. a hazy grey disk with a darker outer zone (or a nebulous white disk with a brighter outer zone). This disk has almost the aspect of a ring, but it is not as clear-cut as in the next two instances. Cover-glass of correct thickness.

Under-corrected Objective. A sharp dark ring (or a sharp bright ring) when the objective is racked down beyond its true focus. A nebulous grey disk with a darker centre (or a milky disk with a brighter centre) when the objective is racked up inside its focus. Too thin cover-glass.

Over-corrected Objective. A nebulous grey disk with a darker centre (or a milky disk with a brighter centre) when the objective is racked down beyond its focus. A sharp dark ring (or a sharp bright ring) when the objective is racked up inside its focus. Too thick cover-glass.

Correcting for Spherical Aberration by Means of the Drawtube

With the exception of the apochromates, most dry objectives are not provided with a correction collar and the spherical aberration must be neutralized by means of the drawtube. The latter is lengthened if the cover-glass is too thin and shortened if it is too thick. It is for these adjustments that a rackwork drawtube is particularly convenient. In practice it is not necessary to ascertain if the cover-glass is too thin or too thick or if the objective is under- or over-corrected as there is a very simple method by which the best image can be easily obtained. This 'automatic' process will be presently described; but a few words may first be said on how to prepare a suitable slide for training the eye, a slide that will show the characteristic appearances as clearly as possible.

In the author's opinion, these conditions are best fufilled by holding a slide over a candle flame until a deposit of soot is obtained. A small fragment of this soot, emulsified in a few drops of xylol, is placed on another slide and mounted in Canada balsam. In such a preparation it is easy to find a particle of carbon of a size suitable for the magnification of the objective

used. In the present instance however, a particle may be sought among the diatom frustules on the slide (grain of dust, fragment of frustule, etc.) and one chosen which is as small as possible while still being distinctly visible.

This particle should be brought to the centre of the field and carefully focused. By means of the fine adjustment, the objective is then slowly raised and lowered and the image of the particle is examined at an equal distance on either side of the true focus. If the appearances are identical, the correction is satisfactory.

Should this not be the case, the microscopist need not trouble to find out if the defect is due to under- or over-correction. The objective is simply moved by means of the fine adjustment, either upwards or downwards, until the *sharp ring* appearance can distinctly be seen. When this has been achieved, the object is brought back into exact focus not by touching the fine adjustment, but by lengthening or shortening the drawtube as the case might require. The correction is once again tested and, if still unsatisfactory, the dark ring is once more brought into view by means of the fine adjustment and the exact focus re-established with the drawtube. This manœuvre is repeated as many times as necessary to obtain perfect correction, i.e. until the same appearances (a hazy disk with a darker outer zone) are noted on either side of the focus.

The whole point of these manipulations is to discover the best working distance of the objective for the particular thickness of cover-glass under consideration, as it will have been noticed that the working distance diminishes when the tube is lengthened and becomes greater if the tube is shortened. This means that, once this optimum working distance has been obtained, it should not be altered. When it is necessary to make *appreciable* changes in the focus, either to study the various planes of a thick object or because the eyepiece has been replaced by another, the adjustments should be made with the *drawtube* and not with the fine adjustment. Here again the rackwork drawtube is extremely useful.

Correcting for Spherical Aberration by Means of the Correction collar

From the appearances previously described, it is easy to determine the degree of correction of an objective. An under-corrected objective means a too thin cover-glass and the correction collar should be rotated clockwise to bring the numbers indicating decreasing thicknesses in front of the fixed pointer engraved on the mount. With an over-corrected objective, sign of a too thick cover-glass, the collar is rotated anti-clockwise to bring numbers corresponding to increasing thicknesses opposite the pointer.

The divisions on the collar are of little practical usefulness however and here, once again, it is unnecessary to ascertain if the objective is under- or over-corrected. Instead of this, the 'automatic' method already described for correcting by means of the drawtube can be employed.

The objective is carefully focused on the object and, by means of the fine adjustment, its out-of-focus image is examined on either side of the true focus. If these images are identical, the objective is properly corrected. If this is not the case, the objective is raised or lowered by means of the fine adjustment until the *sharp dark ring* appears. When this is seen, instead of utilizing the drawtube as previously, the object is brought back into exact focus by turning the *correction collar* in one direction or the other. This process should be repeated until the correction is satisfactory.

As already mentioned, the whole point of these adjustments is to find the exact working distance for the objective which will restore the latter's spherical correction when this has been upset by the wrong cover-glass thickness. Consequently, when this working distance has been found, it should not be altered. If appreciable changes in the focus have to be made, either when examining the different planes of a thick object or when another eyepiece is used, the focusing mechanism should not be employed or the spherical correction will be once more disturbed. Changes of focus should be carried out by rotating the correction collar or by modifying the length of the drawtube.

The correction for the spherical aberration should always be

attended to for all objects examined. It has a very marked influence on the sharpness of the more minute details and the focusing cannot be considered perfect if it has not been done. Any small punctiform detail (grain of dust, cell nucleus, etc.) is suitable for this adjustment provided that it is *in the same plane* as the region to be studied.

A filiform object can also be used. With an under-corrected objective, a fine filament will appear as two sharp dark lines beyond and as a nebulous grey band, darker in the centre, within the point of true focus. The contrary is seen with an over-corrected objective. If the latter is well-corrected, a grey band with darker edges is seen on either side of the focus.

Watson and Sons have placed on the market a very useful Tube Length Corrector computed by Sir Herbert Jackson, F.R.S. This piece of apparatus, which is used in exactly the same way as an objective correction collar, may be mounted between the nosepiece and microscope body or as a part of the body itself. It allows corrections to be carried out easily with all objectives and removes one of the principal disadvantages of high-power Binocular Microscopes in which tube-length adjustment is impossible.

(b) The Condenser

The condenser has already been corrected for slide thickness with the 25 mm. (1 in.) objective (see page 79) and no further adjustment is needed.

6. Regulating the Intensity of the Light

If the manipulations described so far have been carefully executed, the microscope image will be the best that can be obtained. In the case of the *Pleurosigma angulatum*, the frustules should appear *flat* and covered with minute *sharp* black punctae which change to *sharp* white punctae when the focus is slightly modified.

It may happen however that the field is somewhat too bright, causing eye strain and fatigue if work is prolonged. If this should be the case, the light must *not* be toned down by closing the condenser iris, but by interposing a neutral tint or greenish-blue

glass filter in front of the illuminant or in the condenser stop carrier. The Wratten filters are excellent for this, one of the best being their No. 44 as its greenish-blue colour is very restful to the eyesight. For very delicate work, these filters should be of optically worked glass with parallel faces so as not to produce multiple images of the light source.

For visual observation at least, it is rare for the illuminant to be too dim, even with high-power immersion objectives and immersion condensers. If this should be the case however, it is generally due to faulty technique or, in the case of immersions, to bad oil contact between the cover-glass and the objective front-lens or between the condenser and the object-slip.

It is best not to attempt to increase the intensity or the area of the illuminated field by means of a bull's-eye or stand condenser, as any lens interposed between the light source and the substage condenser upsets the corrections of the latter. The stand condenser should be reserved only for photomicrography, where a uniformly illuminated field must be obtained even at a slight sacrifice of the definition, or for work with dark-ground illuminators where great light intensity is of paramount importance.

Technique to be employed with High-Power Oil-Immersion Objectives and an Achromatic and Aplanatic Oil-Immersion Condenser

APPARATUS USED

Light Source

Oil lamp or 60 W. opal electric bulb.

Microscope Stand

Medium or large sized stand as described in Chapter III.

Optical System

Objective: A 2 mm. (1/12 in.) apochromatic oil-immersion of 1·30 or 1·40 N.A., or a 2 mm. semi-apochromatic oil-immersion of 1·30 N.A.

Eyepiece: A 25 mm. compensating ocular × 10.

Condenser: An achromatic and aplanatic oil-immersion condenser of 1·30 N.A.

Mirror

The *plane* mirror, preferably of stainless steel.

Slide to be Examined

Any suitable object mounted in a medium whose refractive index is not lower than that of glass (1·52) or otherwise the numerical aperture of the objective will be correspondingly reduced. A slide of stained *mycobacterium tuberculosis* mounted in cedar-wood oil or Canada balsam.

Cover-glass Thickness

The total thickness of the cover-glass, the mounting medium and the object should not be greater than the working distance of the objective employed—about 0·05 mm. for the 1·40 N.A. apochromate. Apart from this, oil immersions are not affected by variations in the cover-glass as cedar-wood oil has the same refractive index as glass. They can even be used without a cover-glass on suitable preparations.

Slide Thickness

The total thickness, including that of the object and the mountant, should not exceed the focus of the condenser (1·5 mm.); otherwise oil-immersion condensers are insensitive to varying slide thicknesses.

PRELIMINARY MANIPULATIONS

Exactly as described for medium-power dry objectives on page 76.

FINAL MANIPULATIONS

Before focusing the objective and the condenser, a drop of cedar-wood oil should be placed on the under side of the object-slip and another on the condenser front-lens. One should not be afraid of using a fair amount of oil so as to maintain proper contact between the condenser and the slip. The oil will be

spread over a large surface when the slide is moved about in all directions during examination.

The condenser should then be *very slowly* raised for no air bubbles to be formed when the two drops of oil merge into one.

A. PREPARATORY FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

1. Focusing the Objective

The 25 mm. (1 in.) objective is focused as described on page 72.

2. Focusing the Condenser

As described on page 77 for the dry condenser.

It may happen that, just as the image of the illuminant is being focused, a confused shadow is seen to sweep across the field. The latter immediately grows darker and the image of the light source suddenly changes size. This means that the oil film has broken contact between the condenser front-lens and the under side of the object slip owing to the latter being too thin. This can be remedied by building up the slide thickness by affixing one or more cover-glasses to its under surface by means of cedar-wood oil. Good results may also be obtained in some cases by using a more viscous medium, such as golden syrup, in place of the oil, but this is generally not to be recommended.

The condenser may also come into contact with the slide before it can be focused owing to the object-slip being too thick. There is generally no remedy for this except to try bringing the lamp nearer the mirror with the disadvantages that this entails (see page 78).

3. Centring the Condenser

As described on page 78.

4. Correcting the Condenser for Slide Thickness

This is unnecessary with an *oil-immersion* condenser as it is united to the object-slip with a medium having the same refractive index as glass.

B. FINAL FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

1. Focusing the Objective

The focusing of an oil-immersion is not much different from that of a medium-power dry objective, except that special care must be exercised, especially with the 1·40 N.A. lens, owing to the immersion's much smaller working distance.

A drop of cedar-wood oil is placed on the cover-glass and another on the objective front-lens and the coarse adjustment is racked downwards until the two drops merge. Care must be taken that no air bubble is formed between the front-lens and the cover-glass. If this should happen dark streaks will be seen in the field when the objective is focused and the object will appear dark and ill-defined. By examining the back-lens of the objective, the air bubble or bubbles can be easily seen. An air bubble between the slide and the condenser front-lens can be similarly tracked.

To recognize if an air bubble thus observed is between the objective front-lens and the cover-glass or between the slide and the condenser, it is sufficient to raise the objective very slowly by means of the fine adjustment. If the bubble is between the condenser and the slide, no appreciable change in its size or position will be seen. If, on the other hand, the bubble is between the cover-glass and the objective, it appears to grow in size and to move towards the periphery of the back-lens. The microscopist should familiarize himself with all the aspects of the objective back-lens from which, as has already been repeatedly seen, much important information can be learnt.

Immersion objectives can be focused in two ways. While observing from the side, the objective can be carefully lowered by means of the coarse adjustment until the front-lens is almost in contact with the cover-glass. Then, with the eye at the eye-piece, the objective is *very slowly* raised, still with the coarse adjustment, until the object comes vaguely into view. The final focusing is subsequently done with the fine adjustment.

The other method requires some experience. When the first contact takes place between the drop of oil on the cover-glass

and that on the front-lens, one knows that the latter is still *inside* its focus. With the eye at the ocular, the objective can consequently be lowered carefully by means of the coarse adjustment with the knowledge that the object will soon be vaguely glimpsed. When it is first seen, the focusing is finished with the fine adjustment.

This method is quicker, but it should only be attempted if the nature of the preparation is such that there is no fear of falling on an empty field. Otherwise there is the danger of crushing the front-lens down upon the cover-glass. For the same reason, unless one has an exceptionally light touch, it is more prudent when using an objective with a very short working distance, such as the 2 mm. 1·40 N.A. apochromate, or a preparation with a very thick cover-glass, to lower the objective with the *fine adjustment* after the contact of the two oil drops.

Except when special objective changers are used by means of which the optical axes of all the objectives can be made to coincide, it is sometimes difficult to relocate an object with the 2 mm. (1/12 in.) oil-immersion, even though it may have been brought into the centre of the field of the 25 mm. (1 in.) objective. As previously mentioned on page 73, an enlarged image of the object can generally be seen in the objective back-lens, provided that its dimensions are not too minute (minimum diameter about 0·15 mm.), or brought into view by a slight displacement of the object-slip. This image should be brought into the exact centre of the back-lens and the object will afterwards be found in the field when the eyepiece has been replaced and the final focusing completed. The object is sometimes more easily seen in the back-lens if the objective is slightly outside its focus.

2. Focusing the Condenser

As described on page 82.

3. Centring the Condenser

As described on page 84.

4. Regulating the Illuminating Cone

As described on page 84.

5. Correcting the Optical System for Spherical Aberration

(a) The Objective

Homogeneous oil-immersion objectives are united to the cover-glass by a medium possessing the same refractive index as the latter; for this reason it is generally accepted that they are uninfluenced by varying cover-glass thicknesses. We are not entirely of this opinion as we have noticed that the image has sometimes been appreciably improved by a *slight* alteration of the tube-length. Naturally, with preparations mounted in a medium with a different refractive index from that of glass (water, serum, glycerine, Realgar, etc.), correcting for cover-glass thickness recovers all its importance and must be carefully done if the best image is to be obtained.

In most cases however, it will be sufficient to set the drawtube at the length for which the objective has been corrected, except in the cases mentioned above. It is worth while though to verify the tube-length for oneself on a balsam-mounted object as it may not quite correspond to that given by the maker. We have met with objectives supposedly corrected for 160 mm. which, when tested, revealed a difference of as much as 10 mm. above or below that length.

(b) The Condenser

No correction is necessary with an *oil-immersion* condenser.

6. Regulating the Intensity of the Light

As described on page 91.

Technique to be employed with High-Power Oil-Immersion Objectives and an Achromatic and Aplanatic Dry Condenser (0·90—0·95 N.A.) or an Achromatic and Aplanatic Oil-Immersion Condenser used dry

The whole procedure is exactly the same as when using an achromatic oil-immersion condenser immersed, except for the following differences:

B. FINAL FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

(1), (2), and (3), exactly as described for the oil-immersion condenser on page 95.

4. Regulating the Illuminating Cone

As the maximum numerical aperture of a dry condenser is theoretically only 1·0 while that of an oil-immersion objective is 1·30 to 1·40, it follows that the largest cone given by the condenser must be used in its entirety and the iris diaphragm closed as little as possible. The objective back-lens is examined and the iris should not be closed any further as soon as its leaves become visible around the periphery. The iris is closed just a fraction more in the case of a water-immersion objective.

5. Correcting the Optical System for Spherical Aberration

(a) The Objective

As described on page 97.

(b) The Condenser

The condenser is not united to the slip by a drop of cedar-wood oil and consequently it must be corrected for the thickness of the slide as described on page 79, whether it is an achromatic dry condenser or an oil-immersion condenser used dry. If this correction is not carried out, a perfect image will not be obtained and will betray itself, especially in photomicrography, by a certain loss of sharpness and contrast.

Technique to be employed with a High-Power Water-Immersion Objective (2·5—2 mm. F.L., 1·10—1·25 N.A.) and an Achromatic and Aplanatic Dry Condenser (0·90—0·95 N.A.)

The whole procedure is exactly the same as that described on page 74 for medium-power dry objectives, except that the front-lens is united to the cover-glass by a drop of distilled water.

The numerical aperture of water-immersions does not exceed

1·25, even for apochromates, and it is consequently unnecessary to employ an immersion condenser with them. In fact to do so would mean the sacrifice of one of the water-immersion's chief advantages: the ease and rapidity with which they can be used without having to wipe sticky immersion fluid from the surfaces of slides and lenses. They are particularly sensitive to variations of cover-glass thickness and the correction for spherical aberration should be carefully carried out. Most water-immersion objectives are provided with a correction collar for this purpose, but the drawtube may also be used if necessary.

2. OBLIQUE ILLUMINATION

In oblique illumination, instead of filling as much of the objective back-lens as possible with a solid axial cone, only an eccentric portion of the back-lens is illuminated by a light beam inclined upon the optical axis.

Under certain conditions, oblique illumination can increase the resolution of an objective, but the images thus obtained are not for the most part true ones and must be interpreted. The greater visibility of certain details is accompanied by the obscuring of others and the appearances seen do not always correspond to *real* structures as they are often mere diffraction effects. It is not rare for fictitious details to be observed *outside* the object on the opposite side to the light.

Oblique illumination is based upon that postulate of Abbe's theory which claims that the finest details in an object produce the diffracted beams which are the most distant from the direct beam.

If an object, such as a diatom, with a very fine grating-like structure (Fig. 11A) is examined under the microscope, its image will be composed of a direct beam 'a' and of four diffracted beams, 'b', 'c', 'd' and 'e', whose distance from the direct beam will depend on the fineness of the grating (Fig. 12).

If the numerical aperture of the objective employed is so small that it can only embrace the direct beam 'a' (the circle No. 1 represents the back-lens of the objective), the diatom will show no visible structure, as in Fig. 11B. If, however, the direct beam

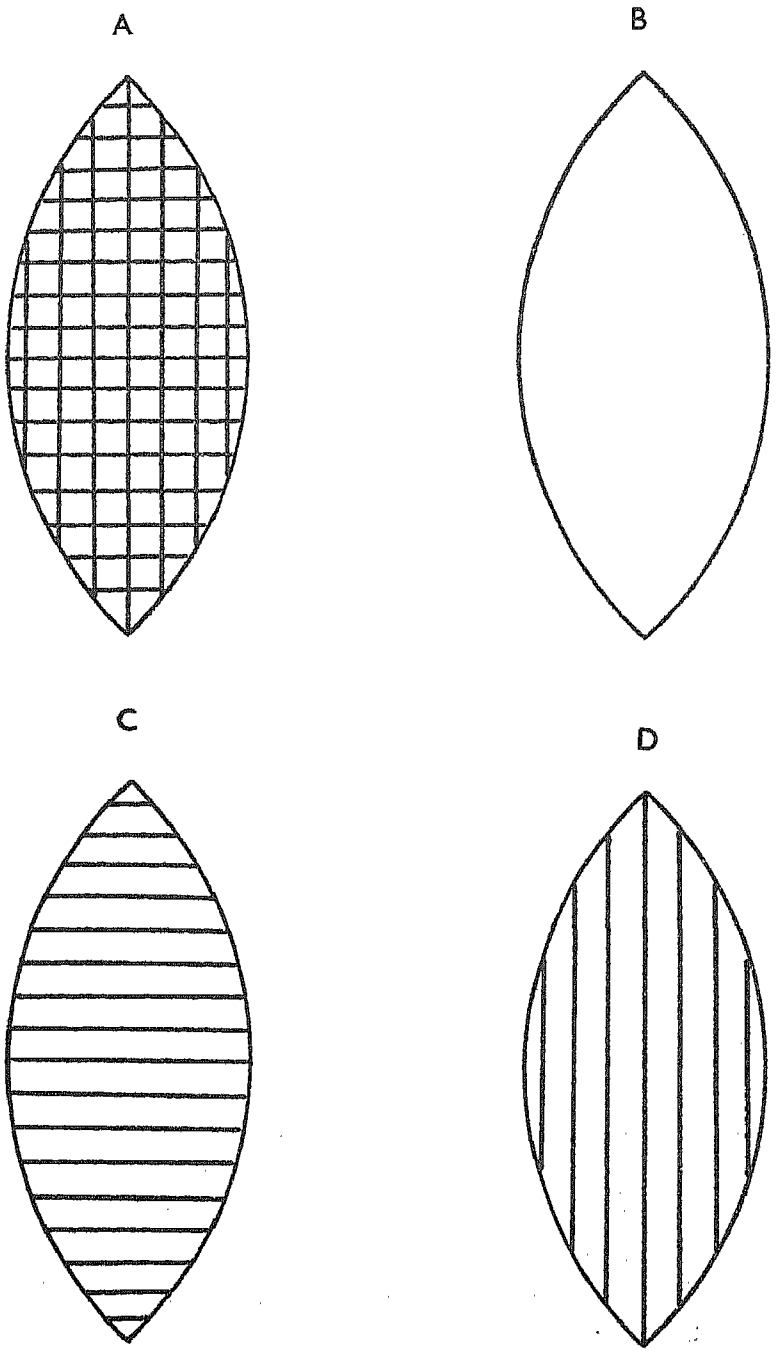

FIG. 11

can be displaced so that it occupies the marginal zone instead of the centre of the back-lens—which can be done by illuminating the object with an oblique pencil—it is evident that the diffracted beam 'c' will be included in the circle. The diatom will then appear to be crossed by horizontal striations as in

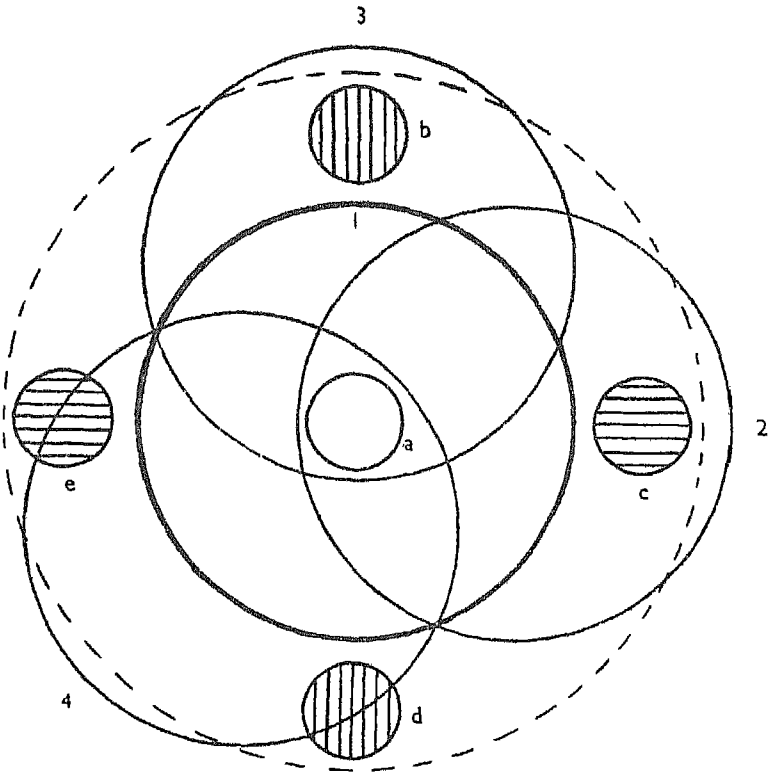

FIG. 12

Fig. 11C. The same object will show longitudinal lines (Fig. 11D) if all or part of the diffracted beam 'b' enters the objective at the same time as the direct beam, as in Fig. 12, circle 3. Finally, if a portion of the two beams 'd' and 'e' accompany the direct beam (Fig. 12, circle 4), then the diatom will reveal its complete grating-like structure.

It should be noted that two fictitious images of one and the

same object have been obtained by using oblique illumination, and even the last picture will be so blurred by effects due to diffraction and to the aberrations of the outer zones of the objective that it will only present an approximate resemblance to reality.

Incomparably better results would have been obtained by using an *axial* illuminating cone and an objective with a numerical aperture large enough to embrace all the diffracted beams, 'b', 'c', 'd' and 'e', as shown by the dotted circle in Fig. 12.

An object with a fairly simple structure was chosen for the

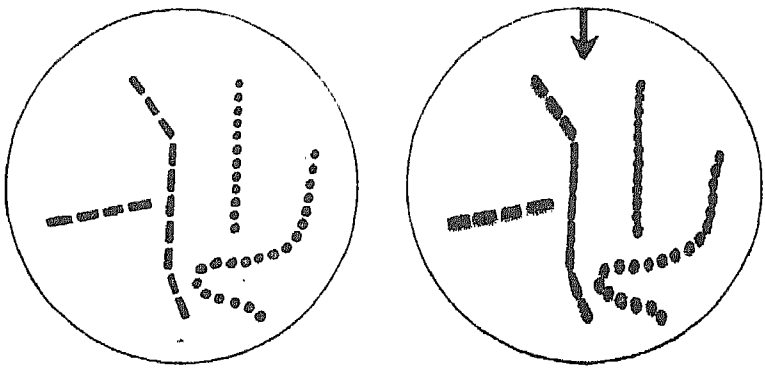

FIG. 13

example just described. False images and other causes of error become increasingly numerous as more complicated structures are considered, especially those whose details do not follow a periodical arrangement.

Most objects studied in medicine and biology belong to this last category and consequently oblique light will be rarely employed. Fig. 13 is given as a warning of what may be observed in a bacteriological preparation examined by oblique light reaching it from the direction of the arrow in the upper quadrant of the drawing. Micro-organisms are rendered unrecognizable and a bacillus or a streptococcus may assume the aspect of a long filament.

With fairly low powers however and objects having a certain relief, good results may be obtained by oblique illumination owing to the artificial intensification of the shadows thus produced. In every case great care should be exercised and the appearances interpreted with caution.

Technique to be employed for obtaining Oblique Illumination

In medicine and biology, oblique illumination may occasionally prove useful, generally with low and medium powers, when very transparent unstained objects are examined.

A sufficient degree of obliqueness can often be obtained by altering the angle of the mirror slightly until sufficient contrast is achieved by intensification of the shadows.

When still greater obliquity is desired, a stop of suitable shape

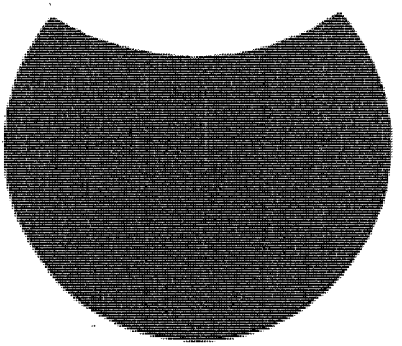

FIG. 14

must be placed in the condenser stop carrier. One of the best shapes is shown in Fig. 14. It can be made of a disc of thin black cardboard; the dimensions of the segment that must be removed depend on the numerical aperture of the objective and the condenser and the degree of obliquity to be obtained in the light beam. Its surface is in inverse proportion to these two factors and can best be ascertained by a few trials. The stop should be placed as near as possible to the inferior focal plane of the condenser and orientated in such a way that the oblique beam

of light is directed at right angles to the details that are to be revealed.

To achieve this, the position of the object relative to the field must be observed. If, for example, the long axis of the object, believed or known to be transversally striated, crosses the field from right to left, the procedure is as follows: The eyepiece is removed and the stop rotated in its carrier so as to illuminate the *right* or the *left* outer zone of the objective back-lens. The eyepiece is then replaced and, if necessary, a small readjustment to the position of the stop will bring out the transverse striae of the object with their maximum contrast.

Oblique illumination must be used with prudence and a certain amount of distrust—this cannot be stressed too often. Whatever the method used, the obliquity of the light beam should never be too exaggerated and no image obtained in this manner should be definitely accepted as a true one unless it can be confirmed by axial illumination.

Annular Illumination

Annular illumination is obtained by employing an axial cone of which the central rays are blocked out by a round stop, shaped as in Fig. 15, placed in the condenser stop carrier.

The diameter of this stop depends on the numerical aperture of the objective and the condenser; it should not be large enough

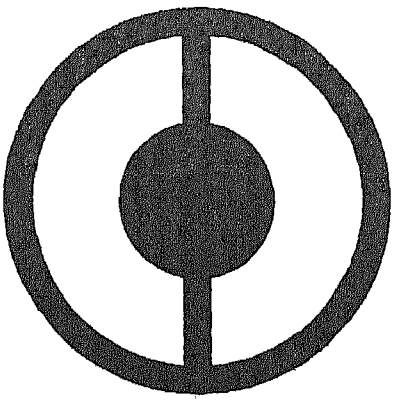

FIG. 15

to produce a *dark ground* by preventing the marginal oblique rays from entering the objective directly.

This form of illumination can sometimes increase the visibility of objects with a periodical structure, such as diatoms, but it has otherwise little practical use in medicine and biology.

CHAPTER V

ILLUMINATION BY DIFFRACTED AND BY REFLECTED LIGHT

ILLUMINATION BY DIFFRACTED LIGHT OR DARK-GROUND ILLUMINATION

Dark-ground illumination is mostly used for the study, in a living and unstained state, of very minute transparent organisms having about the same refractive index as the liquid in which they are swimming.

The technique varies according to whether the objective used has a smaller or larger numerical aperture than about 0·65. With the former a dry achromatic and aplanatic condenser provided with an appropriate stop is sufficient to obtain the required effect; with the latter special immersion dark-ground illuminators are necessary. An Abbe condenser can give a dark ground with objectives up to about 0·40 N.A. and an ordinary immersion achromatic and aplanatic condenser up to about 0·75 N.A.

For satisfactory dark-ground illumination, the light must be directed on to the object at such an oblique angle that none of the rays can enter *directly* into the objective front-lens. The latter must only receive the rays which are diffracted (principally), reflected or refracted by the object, which then seems to shine by its own light against a dark background. The dark-ground condenser must accordingly have a higher numerical aperture than that of the objective with which it is to be employed and the stop must cut off all the rays of lower numerical aperture than that of the objective.

These conditions are easily realized with objectives of low numerical aperture and long working distance (Fig. 16), but it is very difficult to direct the rays at an oblique enough angle for

objectives of high numerical aperture and very short working distance (Fig. 17). It is in these cases that special dark-ground condensers are necessary.

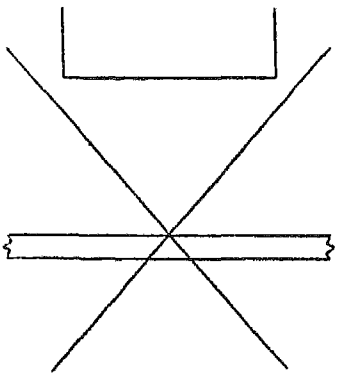

Fig. 16

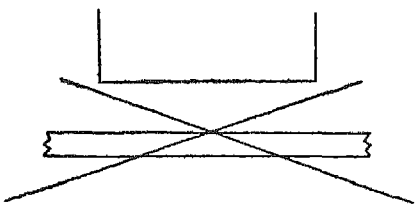

Fig. 17

As most dark-ground work is done with high-power objectives, the description of the technique to be employed will commence with these latter.

Technique to be employed with Immersion Objectives and Dark-Ground Illuminators

The fundamental rules given on page 68 retain all their importance in dark-ground illumination and the goal of all dark-ground technique is to obey them as far as possible. The only points of difference from axial illumination is that the object is illuminated by a hollow cone and that the objective back-lens must appear entirely filled with light.

APPARATUS USED

Light Source

A light source of great intensity is needed for working with dark-ground condensers if the best results are to be obtained. This form of illumination shows up the smallest aberrations of an objective and even the tertiary spectrum of apochromates becomes apparent. Consequently it is often advisable to use

monochromatic filters and these entail a notable loss of light. Again, if transparent objects with a refractive index little different from that of the surrounding medium are to be examined, a very intense light beam is required to make them visible as they deflect only a minute proportion of the rays which fall on them. It is true of course that too bright a light will cause a highly reflective object to be lost in an irradiated haze, but in such a case it is easy to tone down the brilliance of the source by means of appropriate filters.

Owing to the above reasons the oil lamp is not always satisfactory, although very good work can often be done with it. An opal electric bulb is somewhat better, but the three best sources are: The acetylene lamp, the low voltage spiral filament electric bulb and the 'Pointolite' electric bulb. The second illuminant is the simplest and most economical, but the 'Pointolite' is the best for delicate work as it allows the intensity of the light to be modified within very considerable limits by means of suitable filters. The 'Pointolite' electrode presents however a very small luminous surface and a stand condenser—preferably achromatic and aplanatic—will generally be needed to increase the size of the illuminated portion of the microscope field.

A 100 c.p. 'Pointolite' lamp and an achromatic and aplanatic stand condenser of 7 cm. (2 3/4 in.) F.L. are used in the descriptions which follow.

Microscope Stand

Medium or large model as described in Chapter III.

Optical System

Objective: With dark-ground illumination the aberrations of the objective, especially chromatic aberration, become much more evident than with transmitted light. For this reason apochromates or semi-apochromates should, if possible, be employed, preferably the former. The numerical aperture of the objective must be smaller than that of the dark-ground condenser and should not exceed the maximum limit indicated by the maker. The numerical aperture of immersion objectives can be reduced by special funnel stops placed as near as possible

to the back-lens. The best position however for this stop is *between* the component lenses of the objective and far better results are consequently achieved with objectives that have been specially made for dark-ground work than with those whose numerical aperture has been reduced by a removable stop.

In the following description, the objective used is a 2 mm. (1/12 in.) apochromate of 1·20 N.A., or one with an incorporated iris diaphragm by which the numerical aperture can be reduced to that value.

Eyepiece: A compensating ocular of 25 mm. F.L. × 10. In dark-ground work the employment of objectives having a low partial magnification in conjunction with high-power eyepieces is sometimes recommended. It has always seemed to us that the contrary gives better images and that no obvious gain results from using oculars magnifying more than 10 or, at most, 15 diameters.

Condenser: Beck's Patent Focusing Dark-Ground Immersion Illuminator. This condenser has been chosen as an example as it is the one with which the author is best acquainted and has the advantage that it can be focused through slides 0·5 to 1·5 mm. thick. The technique remains the same however with other patterns of dark-ground condenser, except that with these there is less latitude for different slide thicknesses.

Mirror

The plane mirror, preferably of stainless steel.

Slide to be Examined

Any suitable object immersed in a medium having a refractive index of 1·33 or higher. The medium containing the object to be examined must be in *as thin a layer as possible* and should not contain air bubbles or other foreign bodies. If these conditions are not fulfilled, a really black background cannot be obtained owing to the considerable amount of light scattered by objects other than those which are to be studied. When the medium is too thick and the foreign bodies too numerous, this parasitic light may be intense enough to blur the details and make all observation impossible.

Cover-glass Thickness

As near as possible to that for which the objective has been corrected, which is generally about 0·18 mm. Slightly thinner is often better, so as to allow for the thickness of the mounting medium, but, provided that certain limits are not exceeded, the objective can be corrected for cover-glass thickness by means of the drawtube. No correction may be necessary if the mountant has the same refractive index as glass.

Slide Thickness

Preferably not more than 0·5 mm. thick so that the full numerical aperture of the objective can be used as mentioned on page 47.

The object-slip and the cover-glass should be of good quality glass and cleaned with the greatest care as the smallest impurity becomes very obvious in dark-ground work. The surfaces of the slide and especially the cover-glass must not be stained or oxidized as this defect, which can sometimes pass unnoticed with transmitted illumination, produces a more or less conspicuous haze with diffracted light.

PRELIMINARY MANIPULATIONS

1. Set up the microscope and incline it at a comfortable angle.
2. Set the drawtube for the mechanical tube-length for which the objective has been corrected.
3. Screw the immersion objective and a 25 mm. (1 in.) dry achromatic objective on to the nosepiece, rotating the less powerful lens into the optical axis.
4. Place the illuminant at a distance of 250 mm. (10 in.) from the back-lens of the condenser. If the 'Pointolite' is for *alternating current*, it should be turned until one of the two electrodes is directly behind the other so as to obtain a single light source.
5. Adjust the height of the illuminant until the electrode is on the same level as the mirror.
6. Incline the mirror until the light is directed straight up the body-tube, an adjustment that has to be very carefully done. The

best method is to focus the 25 mm. (1 in.) objective and an ordinary dry condenser on to the object on the stage in the usual manner and then tilt the mirror until the image of the light source is brought into the centre of the field. The ordinary condenser is then replaced by the dark-ground illuminator, care being taken not to displace the mirror.

A quicker way is to remove the optical parts and, looking down the empty microscope tube, to incline the mirror until the lamp image is well centred. For greater precision, it is advisable to hold the eye about 30 cm. (12 in.) from the top end of the drawtube.

Once the light beam has been centred with the optical axis of the microscope, the lamp and the mirror *must not again be touched.*

7. Place the dark-ground illuminator into the substage, after having adjusted it approximately for the thickness of the slide

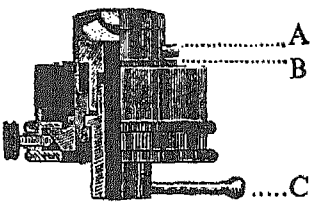

FIG. 18

which is to be used. To achieve this, the slide is inserted between the movable pin 'A' and the ring 'B' (Fig. 18) and slightly nipped by turning the lever 'C'. This, of course, applies only to Beck's illuminator.

FINAL MANIPULATIONS

A. PREPARATORY FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

The preparatory operations are carried out with the 25 mm. (1 in.) F.L. objective.

1. First Centring of the Illuminator

The objective is focused on the upper surface of the front-

lens of the illuminator on which is engraved a small cross or, in some models, one or more concentric rings. This cross or these circles are brought to the exact centre of the field by means of the substage centring screws. The surface of the front-lens must be quite clean and free of all traces of immersion oil, otherwise these engraved markings will not be visible.

When the centring has been carried out, a drop of cedar-wood oil is applied to the front-lens of the illuminator and another on the under surface of the object-slip and the latter is placed on the stage. The illuminator is then racked upwards until the two drops of oil merge, care being taken not to enclose any air bubbles. The presence of an air bubble can be detected by examining the objective back-lens as described on page 95 for transmitted light. The illuminator should be raised until it is almost in contact with the slide.

2. Focusing the Objective

The objective is carefully focused on the object and the region to be examined is brought to the centre of the field.

3. Focusing the Illuminator

The illuminator directs a hollow cone of light on to the object as shown in vertical section in Fig. 19. The object must be placed at the exact apex of the cone 'a' for it to be illuminated. It is evident that if the planes 'b' or 'c' happen to pass through the object, the latter will receive no light. A bright ring with a dark centre will be seen instead in the microscope field (Fig. 19).

If the first centring of the illuminator has been correctly carried out, a bright ring, like the one just described, will be observed in or near the centre of the microscope field. The illuminator should be racked very carefully up or down—by means of the substage *fine* adjustment if the stand is provided with one—until the ring is seen to diminish gradually in size and be replaced by a small bright disk which is really an image of the light source. The position at which this disk presents its smallest diameter is the correct focus of the illuminator.

In the case of Beck's illuminator, as the latter has already been approximately adjusted for the thickness of the slide, the best

focus will have been very nearly achieved when the front-lens almost touches the object slip. It is consequently probable that the bright disk may be observed from the outset. In any case, however, the illuminator should be racked up or down until this disk presents its smallest possible size. A *very slight* readjustment of the lever 'C' (Fig. 18) may be necessary as a final touch.

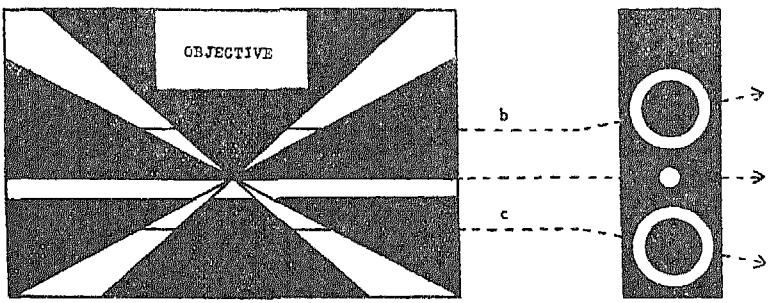

FIG. 19

If the oil has a tendency to break contact between the illuminator and the slide, it is probable that the preliminary adjustment for the slide thickness was forgotten or badly executed and the object slip should once more be inserted between the pin 'A' and the ring 'B'.

The focusing of illuminators which are not adjustable for slide thickness is described on page 117.

4. Second Centring of the Illuminator

The illuminator has already been approximately centred by means of the markings engraved on its front-lens. This centring can be checked by observing if the bright disk previously described is exactly in the centre of the field; it can be brought there, if necessary, with the substage centring screws.

The whole of the back-lens of the objective should appear uniformly illuminated when examined in the usual way after removing the eyepiece. On raising the objective slightly by means of the fine adjustment, a tiny bright spot should appear in the exact centre of the back-lens and, if the objective is raised still

higher, this should be surrounded by a uniformly bright concentric ring.

B. FINAL FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

1. Focusing the Objective

The 2 mm. (1/12 in.) objective is rotated into position and focused as described on page 95.

2. Focusing the Illuminator

Very slight readjustments may perhaps have to be made by means of the substage focusing mechanism or the lever 'C' of the illuminator until the bright area of the field is as sharp and as luminous as possible.

3. Centring the Illuminator

If the bright disk is not concentric with the microscope field, it should be centred with the substage screws.

4. Adjusting the Stand Condenser

If all the manipulations enumerated so far have been correctly carried out, the object, for example a slide of living *Treponema pallida*, should appear crisply and brightly illuminated against a jet-black background. The whole of the microscope field can be satisfactorily lit with some lamps which have a fairly large incandescent surface. This is not however the case with the 'Pointolite' and it may be found that the light is restricted to too small a portion of the field, even with a 2 mm. (1/12 in.) objective. The only solution is to employ a stand condenser to enlarge the image of the light source. This should preferably be an achromatic and aplanatic one so as to introduce as few aberrations as possible to interfere with the performance of the objective and the illuminator. An achromatic and aplanatic stand condenser of 7 cm. (2 3/4 in.) F.L. has been used by the author with very satisfactory results.

The stand condenser should be placed between the lamp and the mirror, at a distance equal to about its own focal length

from the former so as to project a parallel or slightly convergent light beam on to the mirror. The best way of ascertaining the correct position is to place a circle of white paper on the mirror and to move the stand condenser slowly from the lamp towards the mirror until the latter's entire surface is *uniformly* illuminated. It is necessary to make sure that the plane of the stand condenser is parallel to that of the mirror and at right angles to the line joining the centres of mirror and light source. If this is not done the mirror will be more illuminated on one side than the other. The height of the lamp should be adjusted to suit that of the stand condenser. With some light sources a brighter illumination is obtained by projecting an *image* of the lamp on to the mirror.

When the best position has been found for the stand condenser by any of the above methods, it should not again be displaced. The necessary adjustments can be more easily and accurately carried out if the stand condenser can be made to run between wooden slides or along some sort of makeshift optical bench.

Various types of lamp which have been specially designed for dark-ground illumination exist on the market. Some are provided with lenses to project a parallel light beam, some can be employed beneath the stage in place of the mirror, others are even permanently attached to the illuminator. These lamps are very convenient for routine work, but their limited range of adjustment makes them somewhat less suitable for delicate research.

With some types of object, it may be an advantage to reduce the size of the illuminated field by dispensing with the stand condenser.

5. Regulating the Illuminating Cone

The whole of the back-lens of the dark-ground illuminator must be filled with light. Consequently if the substage is of a pattern in which an iris diaphragm is mounted permanently in the substage ring, it should be *fully opened* when the optical part of the dark-ground condenser is placed in position.

6. Correcting the Optical System for Spherical Aberration

(a) The Objective

With dark-ground illumination the smallest errors of spherical correction become much more obvious than with transmitted light. For this reason corrections for cover-glass (and mounting medium) thickness should be executed with the greatest care. This should be done even with oil-immersion objectives whenever the medium surrounding the object has a different refractive index from glass. As oil-immersions are not provided with correction collars, the drawtube must be used as described on page 88.

(b) The Dark-ground Illuminator

No corrections are necessary for slide thickness as the illuminator is an oil-immersion one.

7. Regulating the Intensity of the Light

When the light source is a 'Pointolite' lamp, the illumination will probably be too bright for certain classes of object. This, should it occur, is not only a strain on the eyesight, it also tends to confuse detail by excess irradiation. The light can however be toned down to exactly the right brightness for the object examined by employing neutral tint filters. Beck supplies a very useful accessory, consisting of two neutral tint glass wedges mounted on an adjustable stand, by means of which the desired light intensity can be very accurately obtained.

Dark-ground illumination accentuates the chromatic aberrations of all objectives and even apochromates reveal their tertiary spectrum. It is consequently often advisable to make use of approximately monochromatic light. Amongst the best filters for this purpose are Wratten-Kodak's No. B 58 (green, transmission from 4,600 to 6,000 AU) and No. H 45 (blue, transmission from 4,200 to 5,400 AU).

Technique to be employed with Immersion Objectives of 3·5, 3 and 2 mm. (1/7, 1/8, 1/12 in.) F.L. and Fixed Mount Dark-Ground Illuminators

The technique is exactly the same as that described for Beck's Patent Focusing Dark-Ground Illuminator. Special attention should however be given to the following points:

Slide Thickness

The slide should not exceed the maximum thickness indicated by the maker for any given illuminator or the front-lens will come into contact with the object-slip before it can be focused. The slide should not on the other hand be too thin or the oil film will break contact during focusing. In the latter case however the thickness of the slide can be increased by attaching one or more cover-glasses to its under surface with cedar-wood oil.

Numerical Aperture of the Objective to be Used

With certain forms of dark-ground condenser, such as Watson's 'Cassegrain-Nelson' Illuminator, the whole of the numerical aperture of an objective of 1·40 N.A. can be utilized, provided that the object examined is mounted in a medium with a refractive index of 1·5 or more. This unfortunately rules out most living biological specimens as with aqueous media the numerical aperture of the objective has to be reduced to 1·25 by means of a funnel stop or an internal iris diaphragm.

With most current types of dark-ground condenser, the numerical aperture of the objective must be reduced below 1·0. This can be achieved by means of some form of removable internal diaphragm, such as the funnel stop, but better results are obtained by using objectives which have been specially computed for dark-ground work and have the right numerical aperture.

If the numerical aperture of an objective is too high for the model of dark-ground condenser employed, a portion of the oblique rays can enter directly into the objective front-lens and the background is not completely dark. According to some

authors, it is often easier to distinguish certain details, such as the flagella of bacteria, when the background is a deep shade of grey rather than a dead black.

Focusing the Illuminator

Since fixed mount illuminators have no mechanism for adjusting them to different slide thicknesses, the object-slips should be carefully selected so as to be within the limits of thickness given by the maker.

The focusing of the illuminator is carried out solely by means of the substage focusing adjustment. Apart from this, the technique is exactly the same as that described for Beck's focusing illuminator.

There is no difference in the technique if a water-immersion objective is employed instead of the oil-immersion, except that the correction for cover-glass thickness has to be still more carefully carried out.

Technique to be employed when using a Light-Ground Achromatic and Aplanatic Oil-Immersion Condenser of 1·30 N.A. for Dark-Ground work in conjunction with a Dry 4 mm. (1/6 in.) Semi-Apochromatic Objective of 0·70 N.A.

Very useful dark-ground work can be done with objectives whose numerical aperture does not exceed 0·75 by employing an ordinary achromatic and aplanatic oil-immersion condenser fitted with an appropriate stop placed as near as possible to its posterior focal plane.

The advantages of this method are the great luminosity of the oil immersion condenser and the ease with which one can change instantly from a light to a dark ground and vice versa. To profit from this last advantage, a light source should be chosen that is equally suitable for either method of illumination. As the 'Pointolite' is much too bright for visual light-ground observation, preference should be given to the oil lamp or to the opal electric bulb.

The following formula is used for calculating the diameter of the central opaque stop which will give the best dark-ground illumination with a given objective and condenser.

The numerical aperture of the condenser to be stopped out must be approximately 10 per cent greater than that of the objective.

The diameter in millimetres of the stop is found by multiplying twice the numerical aperture to be stopped out by the focal length of the condenser. In most achromatic and aplanatic immersion condensers, this focal length is about 6 mm. (1/4 in.).

In the present case, with an objective of 0·70 N.A. and a condenser of 6 mm. F.L., the following figures will be obtained:

$$0{\cdot}70 + 0{\cdot}07 = 0{\cdot}77$$
$$0{\cdot}77 \times 2 = 1{\cdot}54$$
$$1{\cdot}54 \times 6 = 9{\cdot}24$$

An opaque diaphragm, shaped as in Fig. 15, with a central stop roughly 9·5 mm. wide, should accordingly be cut out of blackened cardboard and placed in the condenser stop carrier. It would also be advisable to cut out several more stops with diameters varying from about 8·5 to 10·5 mm. and to select the one giving the best results.

If the stop has been correctly shaped, its centre should coincide with the optical axis of the condenser. The simplest way to ascertain if this is the case is to turn the condenser upside down with the stop in position and to close the iris diaphragm gradually. If the disk of the stop remains concentric with the iris opening, it is evident that when the condenser has been centred by means of the iris diaphragm, the stop too will be correctly centred to the optical axis of the objective used.

The illuminant should, as usual, be placed at a distance of 250 mm. (10 in.) from the back-lens of the condenser and, in the case of an oil lamp, the edge of the flame should be turned towards the mirror.

PRELIMINARY MANIPULATIONS

As described on page 76. The object-slip should be united to

the front-lens of the condenser with a drop of cedar-wood oil; the thickness of the slide should not be greater than the focus of the condenser.

FINAL MANIPULATIONS

A. PREPARATORY FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

These are carried out with the 25 mm. (1 in.) objective as described on page 94 for: (1) Focusing the Objective. (2) Focusing the Condenser. (3) Centring the Condenser.

After these manipulations, the stop should be placed in the condenser stop carrier and the iris diaphragm completely opened. A small luminous disk of light will then be seen in the centre of the dark microscope field, and should be made as bright and as small as possible by carefully raising or lowering the condenser. It should finally be brought to the centre of the field with the substage centring screws.

B. FINAL FOCUSING AND CENTRING OF THE OPTICAL SYSTEM

1. Focusing the Objective

The 25 mm. (1 in.) objective is replaced by the 4 mm. (1/6 in.) and focused in the usual way. The luminous area will now be found to fill a much larger portion of the field.

2. Focusing the Condenser

The condenser is very slowly racked up or down until the best results are obtained. If the background is greyish instead of being black, especially towards the margins of the field, the stop is too small. It is, on the contrary, too large if the object does not appear sufficiently illuminated.

It will probably be necessary, at the beginning, to try several stops of slightly different diameters before the best size is discovered to go with the objective employed. Once found however, the width need never be altered. Most optical firms supply

an adjustable stop, known as the 'Traviss' Expanding Stop, with which the most suitable diameter is obtained with the greatest ease.

3. Centring the Condenser

It will only be necessary to bring the luminous area into the centre of the field by means of the substage centring screws. If one wishes at any time to check the exact centring of the condenser, the stop carrier can be swung aside and the condenser centred in the usual manner with the closed iris diaphragm. The latter is then fully expanded and the stop carrier swung back into position.

In all probability the luminous area will not fill the entire field. Its size can be somewhat increased, should this be desired, by turning the flat of the flame towards the mirror. A still larger portion of the field can be illuminated by interposing a stand condenser between the light source and the mirror as previously described for dark-ground illuminators. This however has the disadvantage of diminishing the brightness of the illumination while distributing it over a greater area.

4. Correcting the Optical System for Spherical Aberration

(a) The Objective

Spherical aberration is much more obvious with dark-ground illumination than with transmitted light. Consequently cover-glass correction must be very carefully carried out, either with the drawtube or with the correction collar if the objective is provided with one.

(b) The Condenser

No correction for slide thickness is necessary as the condenser is an immersion one.

5. Regulating the Intensity of the Light

In the present case the light source is an oil lamp or an opal electric bulb, it is consequently very unlikely that the intensity of the illumination will need to be dimmed. Should this be necessary however, it can be done in the usual way by means of neutral tint or coloured filters.

Technique to be employed when using a Light-Ground Achromatic and Aplanatic Dry Condenser of 0·90—0·95 N.A. for Dark-Ground work in conjunction with a Dry Objective of not more than 0·65 N.A.

The technique is exactly the same as that just described for obtaining dark-ground illumination with an ordinary light-ground achromatic and aplanatic oil-immersion condenser.

It goes without saying that no oil is placed between the condenser front-lens and the object-slip. In spite of this, the condenser need not be corrected for the slide thickness as this is achieved automatically with sufficient exactitude as the condenser is focused. This is due to the fact that the focus of the condenser varies with the thickness of the slide; the best results are however obtained if the slides used have the thickness for which the condenser has been corrected.

An ordinary Abbe condenser can give very good results in dark-ground illumination provided that the numerical aperture of the objective employed does not exceed 0·40.

Technique to be employed when using Dark-Ground Illumination for the Examination of Stained Objects

This method, first described by Dr. A. C. Coles (*Parasitology* VII, 1913–1914, pp. 48–49. *Br. Med. Journal*, 27–11–1915, p. 777. *Critical Microscopy*, pp. 70–75), deserves to be more widely known than it apparently is.

It is based on the fact that objects which have been stained with a fluorescent stain (eosin, fuchsin, fluoresceine, etc.) shine out very brightly under dark-ground illumination while those stained blue remain more or less dark. This visibility depends also on another factor: It is greatest when the refractive index of the mountant differs most from that of the organisms examined. The refractive index of bacteria is about 1·55; they will consequently be more difficult to distinguish in Canada balsam, cedar-wood oil (R.I. 1·54) and even water (R.I. 1·33) than in a dry preparation examined with a dry objective.

Dr. Coles advises the use of an ordinary achromatic and aplanatic oil-immersion light-ground condenser provided with a suitable stop. Light source: An oil lamp in conjunction with a stand condenser to project a parallel light beam on to the mirror.

The above condenser presents certain important advantages for this kind of work over dark-ground illuminators: Dark- or light-ground illumination can be instantly obtained by swinging the stop in or out of the optical axis, and it is generally unnecessary to use the condenser immersed.

An apochromatic objective of 8 mm. (1/3 in.) F.L. and 0·65 N.A., partial magnification × 20, should be employed. This objective, together with the advantage of a very wide field, can give a combined magnification of 300 diameters with an eyepiece × 15, which is sufficient to reveal a great many of the pathogenic micro-organisms.

The slides are examined dry, *without any cover-glass*. This allows any organism discovered to be studied in the usual way with an oil-immersion objective and transmitted light after having swung the central stop aside.

To examine a dry preparation without any cover-glass in this manner, the drawtube has to be very considerably extended to correct for spherical aberration. The right length depends on the objective; with a Zeiss 8 mm. (1/3 in.) apochromate in the author's possession the optimum tube-length is 203 mm.

This technique of examining stained specimens by dark-ground illumination is valuable for ascertaining the presence of micro-organisms when these are in very small numbers. Even when they are in a proportion of only one or two per slide, they can be revealed whereas they would probably have passed unnoticed by more usual methods.

The above technique is unfortunately at fault when the preparation contains many highly refractive granulations whose luminosity may mask that of the micro-organisms. Should this happen, it is sometimes possible to reduce the visibility of the granulations compared to that of the micro-organisms and thus render the latter more obvious, by smearing the slide with a very thin film of liquid paraffin (R.I. 1·47).

ILLUMINATION BY REFLECTED LIGHT

Reflected light is utilized for the examination of opaque objects which are too large to be illuminated by diffracted light. The light is directed on the objects in question either: (1) At a more or less oblique angle. (2) Vertically downwards.

The first method can be achieved without special apparatus, either by using the concave mirror of the microscope or an ordinary stand condenser.

In medicine and biology, reflected light illumination is comparatively rarely employed. It can be of value in certain cases however, such as in pathological anatomy when studying whole organs under low powers or in parasitology when examining large parasites which have not been rendered transparent by artificial means.

1. REFLECTED ILLUMINATION WITH THE LIGHT DIRECTED AT AN OBLIQUE ANGLE

It is generally best to illuminate opaque objects with as vertical a light beam as possible so as not to exaggerate the shadows and thus distort the whole aspect of the objects in question. If the centre of the object is higher than its edges, only one half of it will be illuminated if very oblique light rays are used.

It is easy to see however that with high-power objectives having a very short working distance, the light beam must be inclined at a very oblique angle to direct it between the slide and the front-lens and thus illuminate the object.

Most objects are best seen with reflected light when viewed dry and *not covered by a cover-glass* as the latter scatters a good deal of the light and causes a milky haze which confuses the finer details. For the two reasons given above, reflected light is not generally suitable for objectives having a focal length of less than 8 mm. (1/3 in.).

The illuminating beam can be directed on the object either by means of a concave mirror or a converging lens. The mirror is effective with low-power objectives of more than 25 mm. (1 in.) F.L.; the converging lens must be used for higher magnifications.

Technique to be employed when using Objectives of 25 mm. (1 in.) F.L. and longer illuminated by means of a Concave Mirror

The concave mirror of the microscope can be utilized for this method of illumination provided that it is mounted on an independent stand which allows its height and angle to be adjusted at will. The vertical column of this stand must be long enough to allow the mirror to be raised, if necessary, about 100 mm. (4 in.) above the level of the stage.

APPARATUS USED

Light Source

Any illuminant can be used for obtaining reflected light as long as it is not so bright that all detail is confused by excess irradiation; higher magnifications naturally need more light than lower ones. When the concave mirror is employed, the best position for the lamp is 250 mm. (10 in.) to the right or to the left of the stage.

Microscope Stand

Any model.

Optical System

Objective: Any objective having a focal length of 25 mm. (1 in.) or longer.

Eyepiece: An ordinary Huyghenian ocular × 5 or × 10, preferably the former.

Condenser: None.

Mirror

The concave mirror mounted on an independent stand.

Slide to be Examined

Any suitable object, mounted dry.

Cover-glass Thickness

No cover-glass is used.

Slide Thickness

The slide thickness is of no importance. Better results are often obtained by mounting the object on a disk of opaque varnish or black paper to prevent light reaching it from below. Large objects can be examined in a Petri dish or any other suitable receptacle placed on the stage.

PRELIMINARY MANIPULATIONS

The microscope is set up and inclined at a comfortable angle if the nature of the object allows this. The drawtube is set for the correct tube-length and the objective is screwed on to the nosepiece or into the lower end of the drawtube if it has a very long focal length. The lamp is placed at a distance of 250 mm. (10 in.) to the right or the left of the microscope and the concave mirror on its stand is set up about 10 cm. (4 in.) on the opposite side of the stage to the lamp.

FINAL MANIPULATIONS

1. Adjusting the Mirror

The mirror should be placed in such a position that the light from the lamp can reach it without being cut off by any part of the microscope. Its angle, its height on its stand and its distance from the stage are adjusted until the image of the light source concentrated on to the object is as small and as bright as possible. It is generally best for the light rays to be directed as vertically as possible so as not to cause any exaggeration of the shadows.

2. Focusing the Objective

The objective is focused in the usual manner.

3. Regulating the Illuminating Cone

The light entering the objective cannot be accurately regulated, but the quality of the image can be much improved by taking the following precautions: All stray light should be reduced to a minimum by focusing the concave mirror carefully

on the object and restricting the illumination as much as possible to the region under examination. Irradiation haze should be cut down by reducing, if necessary, the intensity of the light source by means of neutral tint filters.

4. Correcting the Optical System for Spherical Aberration

This will scarcely be necessary as only very low-power objectives are employed. It can however be carried out in the usual manner.

Technique to be employed when using Objectives of 8 mm. (1/3 in.) F.L. and longer, illuminated by means of a Converging Lens

Any converging lens of not too short a focus can be used for illuminating opaque objects, but better results are obtained with a plano-convex lens or an achromatic and aplanatic stand condenser.

The technique is very simple with objectives of 12 mm. (1/2 in.) F.L. and longer. The converging lens, mounted on a suitable stand, is placed on the same side of the microscope as the lamp; in the case of the plano-convex lens, the *flat* side is turned towards the illuminant. The lens is first brought close to the stage and then moved slowly away until the smallest and brightest possible image of the light source is concentrated on the object. The rays should be directed as vertically as possible to avoid exaggerated shadows.

If the shadows thrown by the object are too marked owing to a very uneven surface, it may be advisable to illuminate one side of it with the converging lens and the other side with the concave mirror (or another converging lens) or even with a piece of glazed white cardboard.

It is sometimes very difficult with objectives of smaller focal length than 8 mm. (1/3 in.), to insinuate the light beam between the object and the front-lens of the objective owing to the latter's very short working distance. When this is the case, the plano-convex lens must be used and orientated in such a way that its plane surface, turned upwards, is almost parallel with the beam

from the lamp as shown in Fig. 20. The light is concentrated by the convex face and then reflected by the plane face in a

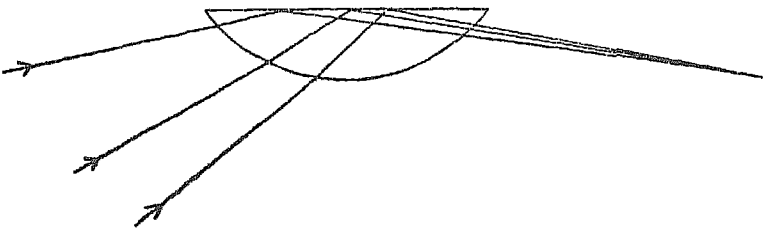

FIG. 20

narrow ribbon of light which is thin enough to pass between the slide and the front-lens of a 6 mm. (1/4 in.) or even a 4 mm. (1/6 in.) objective. (Conrad Beck, *The Microscope*, p. 39.)

2. REFLECTED ILLUMINATION WITH THE LIGHT DIRECTED VERTICALLY DOWNWARDS

It is impossible to illuminate opaque objects with a more or less horizontal light beam when high-power objectives are used. By means of the *Vertical Illuminator* however in conjunction with special objectives having extra short mounts, reflected light can be employed even with oil-immersions. This apparatus is not utilized in medical or biological work and consequently remains outside the scope of this volume.

ERRORS IN MICROSCOPY AND THEIR CAUSES

All errors in microscopy are due, on final analysis, to mistakes of interpretation; that is to say that the microscopist has conceived a false idea of the nature of the object examined.

These errors of interpretation can however be divided into two categories:

A. Errors of interpretation due to faulty microscopical technique, when the microscopist has not been able to obtain an image of the object which corresponds to reality.

B. Errors of interpretation *per se*; when the microscopist has

obtained a correct image of the object, but has not been able to understand what he has seen.

It is, of course, taken for granted that there has been no gross mistake in *preparing* the object for examination (section cutting, fixing, staining, clearing, etc.). Otherwise a third category of causes of error would be needed to include those due to faulty preparing and mounting.

A. Errors of Interpretation Due to Faulty Microscopical Technique

Most faults in microscopical technique can be traced back to four principal causes:

1. Bad centring of the optical system.
2. Bad focusing of the condenser.
3. Bad regulation of the illuminating cone.
4. Bad correction for spherical aberration.

1. Bad Centring of the Optical System

Bad centring of the condenser with the optical axis of the objective affects the image adversely in various ways: The back-lens of the objective is not uniformly illuminated, thus producing a certain degree of oblique lighting with its accompanying disadvantages (see page 102). The corrections of the objective for spherical aberration are upset with bad effects on the sharpness of the image. The numerical aperture of the objective is not fully utilized and its resolving power is consequently diminished.

2. Bad Focusing of the Condenser

If the condenser is not accurately focused, the objective back-lens cannot be entirely filled with light and its numerical aperture is not fully utilized. This can be proved by examining the objective back-lens. The latter is completely illuminated when the condenser is at its exact focus (it is understood that the numerical aperture of the condenser is suitable for that of the objective) as in Fig. 7B. If the condenser is racked up too much, two dark patches make their appearance (Fig. 7C), whilst if it is racked down the outer zones of the back-lens are insufficiently illuminated (Fig. 7A).

3. Bad Regulation of the Illuminating Cone

This is one of the mistakes which is most frequently met with. Inexperienced microscopists have nearly always a tendency to close the condenser iris diaphragm too much so as to obtain greater contrast and make transparent objects more visible. The results of this practice are most misleading. It is true that coarse details become more apparent, but, owing to the reduction of the objective's numerical aperture, the finer details disappear completely. The remaining details often become unrecognizable owing to diffraction effects. Micro-organisms appear to possess capsules or flagella which have no real existence, the spaces between certain structures are blocked out, fine lines become broadened or run together. In fact the whole image—though it may seem at first sight to be bolder and more contrasting—is found on more careful observation to be blurred and ill-defined.

Closing the iris diaphragm during the examination of very minute and transparent objects cannot reveal their true aspect and can only afford some indications as to their presence or absence, their mobility and their size, the latter much exaggerated by diffraction effects. Even under the best of conditions, the information thus obtained cannot always be relied upon and should only be accepted with the greatest caution.

A mistake less frequently encountered—as nine times out of ten the light sources used for visual observation are too bright—is a too widely opened iris diaphragm. In this case the image is swamped in an excess of light and the finer details are lost or become dim and blurred.

4. Bad Correction for Spherical Aberration

(a) The Objective

It has already been repeated many times in the preceding chapters that if the objective is not properly corrected for the thickness of the cover-glass, its definition is impaired and the details appear more or less ill-defined.

Another disadvantage can also result: According to Abbe's theory, the marginal zones of the objective are responsible for the image of the finer details of the object examined, while the central zones supply the image of the coarser details. In a poorly

corrected objective, the foci of its marginal and central zones are not in the same plane. Consequently if a perfectly flat object is viewed with an over-corrected objective, the foci of the finer details will fall *above* those of the outlines and the coarser details; the microscopist will think that he is looking at a *convex* object. The contrary will occur with an under-corrected objective and the microscopist will imagine that he is observing a *concave* structure. It is unnecessary to stress the errors that might result from this interpretation.

(b) The Condenser

If the condenser is not properly corrected for the thickness of the slide, its numerical aperture is reduced and it may be difficult or impossible to fill the back-lens of the objective with light. Thus the numerical aperture of the latter will be also correspondingly reduced.

B. Errors of Interpretation *per se*

It would be an impossible undertaking to make out a full list of all the errors which might be included in this category. We shall content ourselves by mentioning some of the commonest, including certain foreign bodies which, as they are often in suspension in the surrounding atmosphere, can sometimes be encountered in the most carefully made slides and which may perhaps mislead the unwary microscopist.

1. Presence in the Slide of Substances Having a Different Refractive Index from the Mounting Medium

Air bubbles. It is not rare in blood smears to see in the centre of some of the red blood corpuscles a minute air bubble which might be mistaken for a Malaria parasite. Fat globules. Water globules in resinous mounting media. Droplets of water vapour condensed on the under side of the cover-glass in dry mounts.

2. Presence of Foreign Substances on the Surface of the Slide or the Cover-glass

Grains of dust. Finger marks. Traces of glycerine, cedar-wood oil, Canada balsam or other mounting media. Cracks, scratches or oxidation of the glass surfaces.

3. Presence in the Slide of Foreign Bodies Originating from the Atmosphere, or from the Patient's Food in the Case of Sputum or Faeces Examinations

From the mineral kingdom: Dust, soot, fibres of asbestos, etc.

From the vegetable kingdom: Starch grains from potatoes, wheat, oats, rice, leguminous vegetables, etc. Fibres of linen or cotton. Wood cells. Hairs, cellular tissues and spiral vessels of various plants. Pollen, spores of truffles and mushrooms. Various moulds.

From the animal kingdom: Human hairs, hairs from domestic animals and from clothing; wool, silk, furs, etc. Fragments of down and feathers, which can sometimes be mistaken for lymphatic vessels. Hairs and scales of butterflies, moths and other insects.

It might also be worth while adding to the above list:

4. Spontaneous Movements and Changes Due to Physical Causes

Brownian movement or pedesis; a spontaneous movement of very minute particles in a liquid medium, which may be mistaken for the active displacement of living organisms. Movements in the object or slide due to capillary attraction or to desiccation. Changes in the object due to osmosis; certain structures may swell or shrink if the fluid contained in their cells is more or less dense than the surrounding medium.

Formation of precipitates, deposits or crystals in the object or the mounting medium.

CHAPTER VI

BINOCULAR MICROSCOPES

The microscopes described so far in this book have been of the monocular type. Binocular microscopes however exist which have the advantage of allowing both eyes to be used simultaneously, thus lessening ocular fatigue, and of giving a *stereoscopic* image of the object examined which can thus be seen in its true aspect.

Binocular microscopes can be divided into two classes:

1. Paired objective binocular microscopes. 2. Single objective binocular microscopes.

1. PAIRED OBJECTIVE BINOCULAR MICROSCOPES

These microscopes are represented by the Greenough Binocular which is too well known for any description to be necessary in the limited compass of this volume.

The Greenough Binocular gives true stereoscopic vision, but unfortunately it cannot be used effectively with magnifications exceeding about 150 diameters.

2. SINGLE OBJECTIVE BINOCULAR MICROSCOPES

Single objective binocular microscopes differ from the preceding inasmuch as they can be employed with the highest powers and even with oil-immersion objectives.

The stereoscopic vision which they afford is to a great extent illusory, but under certain conditions, they can furnish an image corresponding fairly well to reality and thus give valuable information on the true aspect of the object examined. The images thus obtained must however be accepted with prudence as relief is often much exaggerated, especially with high magnifications, or confused by pseudoscopic effects.

Two patterns of single objective binocular microscopes can

be met with: In the first the light beam from the objective is *divided* into two halves by the edges of two superimposed prisms. In the second the beam is *duplicated* by means of two prisms whose contacting surfaces are coated with a semi-transparent silver film.

The first pattern should be avoided as, when the light beam is divided into two, the numerical aperture of the objective is also halved. The second pattern is by far the best and stereoscopic vision can be obtained with it if the distance between the optical axes of the two eyepieces is so adjusted as to be slightly less than the observer's inter-pupillary distance.

It should be noted however that this adjustment has the effect of cutting off the light from the inner half of each of the two images of the objective back-lens which can be seen reflected in the prisms when the two eyepieces have been removed. This contravenes two of the fundamental rules for obtaining true images of any object examined, i.e. that the circular shape of the objective lenses must not be impaired and that the back-lens should be uniformly illuminated. From this alone it will be seen that the appearances observed must always be accepted with some prudence.

If the distance between the optical axes of the two eyepieces is identical with the inter-pupillary distance no steroscopic effect is obtained, but there is the important advantage that both eyes can be employed at once. Definition however is not quite as good as with monocular microscopes and these are still the best for all very delicate work.

When the distance between the eyepieces is greater than the inter-pupillary distance *pseudoscopic* vision results in which all reliefs appear to be replaced by hollows and vice versa.

Many optical firms construct their single objective binocular microscopes with parallel bodies, others have them converge at an angle of about four degrees from the vertical. It is difficult to say which is the best form; the author prefers the parallel bodies, but many microscopists find the converging form easier to work with.

Technique to be employed when using Binocular Microscopes

The technique to be employed with binocular microscopes is the same as that already described for monoculars. Special attention should however be given to the following points:

1. The distance between the optical axes of the two eyepieces should be very carefully adjusted. It should be very slightly less than the inter-pupillary distance if stereoscopic effect is desired.

2. The two eyes should be focused separately as their sight is very rarely identical. To achieve this, the eyepiece tube provided with a knurled ring for independent focusing should first be noted. If this is, for example, on the left side, then the right eye alone should first be used and the objective focused on a suitable object by means of the stand's focusing mechanism. When this has been done, the stand's focusing mechanism is not again touched, but the left eye is focused by itself by using the knurled ring on the eyepiece tube. This ring bears a numbered scale by means of which the same adjustment can be quickly reset on subsequent occasions.

3. The illuminating cone should be adjusted in the usual manner by opening or closing the condenser iris. An image of the objective back-lens can be seen reflected in the prisms by removing either of the eyepieces. The light source should be about twice as bright as with monocular microscopes. It is *very important* to equalize the illumination in both fields by carefully adjusting the angle of the mirror.

4. With some models of binocular microscope, the position of the object appears to have a marked influence on the stereoscopic effect. Elongated objects stand out in stronger relief if their long axis crosses the microscope field *horizontally* rather than vertically. Round objects are unaffected unless their principal details run in one direction only.

5. Except when working with very low powers, use should be made, as much as possible, of homogeneous immersion objectives or of objectives provided with a correction collar as binocular microscopes have no drawtube for carrying out cover-glass correction.

This was once one of the most serious objections to high power binoculars, but it can now, fortunately, be overcome by means of Sir Herbert Jackson's Tube-Length Corrector (Watson and Sons) which is a sort of correction collar which can be permanently mounted as part of the microscope body or between the latter and the nosepiece.

Too powerful eyepieces should not be used; the 50 mm. F.L. × 5 and the 36 mm. × 7 give the best results.

ADVANTAGES OF BINOCULAR MICROSCOPES

Binocular microscopes render very valuable services because not only do they lessen ocular fatigue and strain during long spells of work, but the stereoscopic image which they give can very materially help in the examination and interpretation of certain classes of object. They cannot however take the place of monocular microscopes for the observation of very fine detail. For this reason the most complete research stands are usually provided with interchangeable monocular and binocular bodies to suit any kind of work.

CHAPTER VII

METHODS FOR RECORDING OBSERVATIONS

When recording observations, the following information is required concerning the object under examination:

1. Its dimensions: (a) Thickness. (b) Length and width.
2. Its shape.
3. Its position on the slide.

1. METHODS FOR DETERMINING THE DIMENSIONS OF AN OBJECT

(a) Thickness

The thickness of an object seen under the microscope can be measured by means of the divisions which are generally to be found on one of the fine adjustment milled heads. The lowest and the topmost planes of the object are focused in succession and the vertical movement of the objective front-lens is read off on the divided scale, each division of which corresponds to a certain length (generally 1/500 mm.) indicated by the maker.

The figure thus obtained is then multiplied by the refractive index of the medium surrounding the object to find the real thickness of the latter.

(Example: To calculate the thickness of an object mounted in distilled water—R.I. 1·336. A displacement of 0·04 mm. has been read on the fine adjustment milled head scale. This figure is multiplied by 1·336 and the value 0·053 mm. is obtained. This is the real thickness of the object.)

In short, the real thickness of an object is found by multiplying the apparent thickness read from the fine adjustment scale by the refractive index of the medium by which the object is surrounded.

This rule is not upset by the presence of a cover-glass between the object and the objective as the effects produced by it affect

all planes of the object equally. The final figure is consequently not modified.

(b) Length and Width

The *eyepiece micrometer*. The easiest and quickest way to measure the linear dimensions of an object is to employ an eyepiece micrometer. This apparatus, in its simplest form, is just a disk of clear glass ruled with a scale of 10 mm. divided into 100 parts. It is dropped into the eyepiece mount so that it rests on the inner diaphragm in such a way that the ruled scale is directed downwards; a sharp and magnified image of this scale will then be seen in the microscope field.

Before using the eyepiece micrometer, the exact value of a division must first be ascertained for each objective. To achieve this a *stage micrometer* is placed on the stage and carefully focused; the number of divisions of the latter corresponding to one division of the eyepiece micrometer is then counted. If each division of the stage micrometer employed equals 0·01 mm. and if 5 of these divisions correspond to 1 division of the eyepiece micrometer, it is evident that this division represents 0·05 mm. or 50 microns (50μ).

Linear dimensions can now be measured by means of the eyepiece micrometer without any further reference to the stage micrometer being necessary. An object, for example, whose apparent length corresponds to 3 divisions of the eyepiece micrometer would have a real length of:

$$0{\cdot}05 \times 3 = 0{\cdot}15 \text{ mm.}$$

If a division of the eyepiece micrometer does not correspond to an exact number of divisions of the stage micrometer, the greatest number of divisions of the eyepiece micrometer which equal a whole number of divisions of the stage micrometer should be taken and their mean value ascertained. In fact it is advisable to do this in any case so as to reduce to a minimum any errors due to slight variations between the individual divisions of either of the two micrometers. If 10 divisions of the eyepiece micrometer correspond, for example, to 44 divisions of the stage micrometer, then each division of the former equals 0·044 mm.

When calibrating the eyepiece micrometer with a high-power objective, the divisions of the stage micrometer present an appreciable width. For this reason the divisions of the eyepiece micrometer should always be made to coincide with either the right or the left margin of the stage micrometer divisions so that the final results may be as accurate as possible.

When an oil-immersion objective is being calibrated, it should be used dry, as any cedar-wood oil applied to the stage micrometer will cause the divisions to disappear if these have been engraved on the glass as is usually the case.

Finally, it should not be forgotten that the values found as described above apply only to the eyepiece, objective and *tube-length* with which they have been obtained. If the micrometer is transferred to another eyepiece or if the tube-length is altered, the whole calibration must be repeated.

The *camera-lucida*. The object to be measured is roughly drawn on a sheet of paper by means of a camera-lucida or drawing eyepiece of any pattern. The slide is then replaced on the stage by the stage micrometer and the image of its graduated scale is traced in a corner of the drawing. It is then easy to ascertain the exact size of the object by means of a pair of compasses.

This method is more accurate than the previous one when measuring the length of a curved, spiral-shaped or irregularly looped object. In these cases it is often helpful to follow the outlines of the object in question with a piece of thin cotton, slightly damped, and to compare the length thus obtained against the scale reproduced on the drawing. This method is independent of the tube-length and of the objective and ocular employed.

The *mechanical stage*. If the microscope stand is fitted with a well-constructed mechanical stage provided with graduations and verniers, fairly accurate measurements can be made of objects of relatively large size whose dimensions exceed those of the microscope field. To achieve this, a stage micrometer is placed on the mechanical stage and the left hand end of the divided scale is brought into contact with the left margin of the field. The vernier is read and the stage micrometer is then dis-

placed by means of the stage mechanism until its right hand end reaches the left margin of the field. By re-reading the vernier, it is easy to calculate how many stage graduations correspond to a stage displacement of one millimetre. It is advisable to recommence this operation several times at different points of the mechanical stage's run and to take a mean of the figures thus obtained. Once the figures which correspond to a displacement of one millimetre are known, they can be made to show the length of any object on the stage by bringing first its left and then its right end in contact with the left margin of the field.

2. METHODS FOR DETERMINING THE SHAPE OF AN OBJECT

The shape of an object is recorded either by drawing or by photography.

DRAWING WITH THE MICROSCOPE

Drawing Apparatus

To obtain the best results from camarae lucidae and all forms of drawing apparatus, two fundamental rules must be obeyed:

1. The illumination of the microscope field and that of the drawing pencil and paper must be more or less equal.
2. The plane of the drawing paper must bear a certain definite relation to that of the microscope stage (differing according to the apparatus used) to prevent distortion of the drawing.

1. Adjusting the Illumination

The first thing is to ascertain whether the illumination of the microscope field or that of the drawing paper is too bright. If the field is too bright, the pencil point is indistinct or invisible. This can be remedied by diminishing the luminosity of the field by neutral tint filters placed in the condenser stop carrier or in front of the light source. In certain cases the same result is obtained by closing the condenser iris *very slightly*, but this method is rarely to be recommended as it confuses the image and produces misleading diffraction effects. An alternative method

is to increase the lighting of the drawing paper, and for this an auxiliary lamp is often useful.

If the pencil and paper are well seen, but the details of the microscope field are dim and indistinct, it means that the latter is not sufficiently illuminated. The objective should be given more light by using a brighter lamp or a more powerful substage condenser. Alternatively, the illumination of the drawing paper can be diminished by means of a suitable screen. Most patterns of camera lucida are fitted with several small neutral tint glasses which can be interposed in the path of the light rays coming from the paper to tone down their luminosity before they reach the observer's eye.

When the brightness of the microscope field and the drawing paper has been well balanced, the details of the microscope image and the pencil point are both distinctly seen simultaneously. It is generally the microscope field that is too bright with low-power objectives and the other way round with high magnifications. For this reason high-power oculars should not be employed with high-power objectives so as to avoid undue loss of light. Eyepieces $\times$ 3 or $\times$ 5 generally give the best results.

It may happen that the microscopist can see the microscope image clearly, but finds that the pencil point remains indistinct although the lighting is correct. In most cases this means that the distance from the eyepiece to the drawing paper is not suitable for the observer's eyesight. The distance of normal vision is 250 mm. (10 in.) and it is at this distance from the drawing apparatus that the paper must be placed to be well seen and superimposed on the image of the microscope field without accommodation strain and fatigue. If the observer's sight is not normal, the paper should be placed at the distance which he finds most comfortable; nearer than 250 mm. if he is shortsighted and further if he is longsighted. In extreme cases a special correcting lens will have to be fitted to the camera lucida.

2. Precautions for Preventing Distortion of the Image

For preventing distortion of the microscope image, the surface of the drawing paper should be quite flat and it should lie

in the correct plane. With most camerae lucidae this plane must be parallel with that of the microscope stage, but in some models it may even be at right angles to the latter. An adjustable drawing table is very useful for obtaining the correct angle.

It is very simple to verify if the paper lies in the correct plane for preventing distortion as it is sufficient to mark the horizontal and vertical diameters of the field as seen on the paper and to ascertain if these two lengths are equal. If the circumference of the field is a perfect circle, the proportions of the drawing will not be distorted. Some camerae lucidae magnify the marginal areas of the microscope image slightly more than the central region; with these models it is advisable only to use the central portion of the field for drawing.

It must not be overlooked that camerae lucidae can only be used for setting down the outlines and the principal details of an object. The drawing must be completed by removing the drawing apparatus and filling in the finer details from direct observation.

The Squared Eyepiece Micrometer

This accessory consists of a disk of clear glass on which a regular grating has been ruled, each square of which is 1 mm. wide. The disc is dropped into an eyepiece, preferably × 5, with the ruled surface downwards.

For drawing, a sheet of squared paper is used and the details seen in each square of the microscope field are copied in the corresponding square of the paper. This method is one of the simplest and quickest for those who already possess some notions of free-hand drawing.

PHOTOMICROGRAPHY

It would be impossible, within the limits of this volume, to give a detailed description of the various methods used in photomicrography and the reader is referred to the many specialized works on this branch of microscopy. A few pages however on the technique of lighting in photomicrography may not perhaps

be out of place as this all-important subject is often insufficiently dealt with in some text-books.

The aims to be attained in photomicrography are the following:

1. The whole of the microscope field must be uniformly illuminated, with as little sacrifice as possible of the optical quality of the image.

2. Only the area of the object included in the field to be photographed should be illuminated. This reduces to a minimum the stray light that might enter the objective and diminish the sharpness of the image.

3. The whole of the back-lens of the objective must be uniformly filled with light so that its full numerical aperture may be utilized. It is, of course, understood that the usual 3/4 cone is employed for the same reasons as those given for visual observation.

The following methods are based on those described by Dr. Duncan J. Reid, 'The Principles of Illumination in Photomicrography' in the *Journal of the Photomicrographic Society*, Vol. X, No. 1, Feb. 1921.

TECHNIQUE OF ILLUMINATION IN PHOTOMICROGRAPHY

The first condition for securing a good photomicrograph is to obtain as perfect a *visual image* as possible by the methods already described in this volume.

A fairly intense illuminant (acetylene lamp, 'Pointolite', etc.) must be employed, especially with high magnifications, to cut down exposure time to a minimum.

As light sources with a high intrinsic brightness have usually a very small luminous surface, a stand condenser will be needed to illuminate the whole of the field uniformly. This should be preferably an achromatic and aplanatic one; the stand condenser used by the author, with very satisfactory results, has a diameter of 6 cm. (2 3/8 in.) and a focal length of 7 cm. (2 3/4 in.).

Technique to be employed when using the Stand Condenser. Photomicrography with High-Power Objectives

APPARATUS USED

Light Source

100 c.p. 'Pointolite' lamp.

Microscope Stand

Medium or large model. Whenever possible, the stand should be inclined to the horizontal and the light from the lamp directed straight into the substage condenser without the intermediary of the mirror.

Optical System

Objective: A 2 mm. (1/12 in.) apochromatic oil immersion objective of 1·30 N.A.

Eyepiece: A compensating ocular of 25 mm. F.L. × 10.

Condenser: An achromatic and aplanatic oil immersion condenser of 1·30 N.A.

Mirror

The plane mirror, preferably of stainless steel. The mirror is dispensed with if the microscope is used horizontally.

Stand Condenser

Achromatic and aplanatic of 7 cm. (2 3/4 in.) F.L.

Slide to be Photographed

The refractive index of the mounting medium should not be less than 1·54 or the numerical aperture of the objective will be correspondingly reduced.

1. Finding the Correct Position of the Stand Condenser

The best visual image should be first obtained after having temporarily reduced the intensity of the light source by means of an adequate filter.

After all the necessary adjustments have been thus made, the opening of the substage condenser iris should be carefully measured (and found, for example, to be 16 mm. in diameter). The incandescent surface of the 'Pointolite' electrode is about 4 mm. in diameter and an image of it, large enough to fill the diaphragm opening, should be projected on the condenser iris if the best results are to be obtained. This projected image should be preferably slightly larger than the iris opening so that the region which falls on the actual aperture might have a uniform brightness.

The image of the 'Pointolite' electrode must consequently be magnified four times ($16 \div 4 = 4$) or, better still, five times to fill the iris opening and allow for the overlap.

It will now be necessary to find the positions which should be given to the lamp and the stand condenser for the above magnification to be achieved. These data are obtained from the following formula:

If 'x' represents the product of the focal length of the stand condenser multiplied by the magnification to be obtained, then x + the focal length of the stand condenser equals the distance of the stand condenser from the substage condenser iris.

The distance thus found divided by the magnification to be obtained gives the distance from the stand condenser to the lamp.

The sum of these two distances equals the distance from the lamp to the substage condenser iris.

In the present example:

The focal length of the stand condenser is 7 cm.

The magnification to be obtained is 5; consequently

$$7 \times 5 = 35 \qquad 35 + 7 = 42$$

$$42 \div 5 = 8{\cdot}4$$

$$42 + 8{\cdot}4 = 50{\cdot}4$$

This means that to obtain the desired results, the stand condenser must be placed at a distance of 42 cm. from the substage condenser iris and 8·4 cm. from the lamp. The distance from the lamp to the substage condenser iris is 50·4 cm.

2. Regulating the Illumination of the Microscope Field

It is now necessary to make sure that only the area included in the microscope field is illuminated and not any other parts of the object. For this an *auxiliary iris diaphragm* mounted on an independent stand will be needed. This auxiliary iris is placed between the stand condenser and the substage condenser at a distance of 250 mm. from the latter. It is the opening of this auxiliary iris which will now be used as a light source as far as the illumination of the microscope field is concerned.

The auxiliary iris diaphragm is first almost completely closed and its image is carefully focused in the plane of the object on the stage by means of the substage focusing mechanism. The auxiliary iris is finally slowly opened until its margins just frame the area of the field that is to be photographed. This, of course, may include the whole field if necessary.

During all these manipulations the centres of the lamp electrode, the stand condenser, the auxiliary iris diaphragm and the substage condenser (if the microscope is horizontal) should all be on the same optical axis. This can best be achieved by aligning the various accessories on an optical bench or even between home-made slides of wood or metal. If the mirror is used, its centre should be in the same optical axis as the other accessories. This may entail raising the microscope on some sort of block or support.

PHOTOMICROGRAPHY WITH MEDIUM-POWER OBJECTIVES

Optical System

Objective: A 4 mm. (1/6 in.) semi-apochromatic dry objective of 0·80 N.A.

Eyepiece: A compensating ocular of 25 mm. F.L. × 10.

Condenser: An achromatic and aplanatic dry condenser of 0·90 N.A.

After having carefully adjusted the microscope for visual observation, the opening of the substage condenser iris is measured and found to be, for example, 10 mm. in diameter. The image of the light source must consequently be magnified

2·5 times (10 ÷ 4 = 2·5) or rather 3 times to fill the opening and allow for the overlap.

The distances of the lamp and the stand condenser are calculated by means of the formula and found to be:

$$7 \times 3 = 21 \qquad 21 + 7 = 28$$
$$28 \div 3 = 9{\cdot}3$$
$$28 + 9{\cdot}3 = 37{\cdot}3$$

The lamp should consequently be placed at a distance of 37·3 cm. from the substage condenser iris and the stand condenser 9·3 cm. from the lamp.

As in the first example, the auxiliary iris diaphragm should be placed at 250 mm. from the substage condenser iris. All the other manipulations are the same.

PHOTOMICROGRAPHY WITH LOW-POWER OBJECTIVES

Optical System

Objective: A 12 mm. (1/2 in.) achromatic objective of 0·30 N.A.

Eyepiece: A Huyghenian ocular of 50 mm. F.L. × 5.

Condenser: An achromatic and aplanatic dry condenser of 0·90 N.A. It may be necessary to use the condenser with the front-lens removed to illuminate the whole of the field.

The opening of the substage condenser iris is measured and found to be, for example, 5 mm. in diameter. The image of the illuminant must consequently be magnified 1·25 times (5 ÷ 4 = 1·25) or, preferably, twice to allow for the necessary overlap.

The following figures are obtained by means of the usual formula:

$$7 \times 2 = 14 \qquad 14 + 7 = 21$$
$$21 \div 2 = 10{\cdot}5$$
$$21 + 10{\cdot}5 = 31{\cdot}5$$

i.e. The lamp should be placed at a distance of 31·5 cm. from the substage condenser iris and the stand condenser 10·5 cm. from the lamp.

The auxiliary iris must be brought as close as possible to the

stand condenser so that its distance may not differ too much from the correct one of 250 mm.

PHOTOMICROGRAPHY WITH VERY LOW-POWER OBJECTIVES

Objective: A 50 mm. (2 in.) achromatic objective.

If the method just described is employed, it will probably be found that the whole of the microscope field cannot be uniformly illuminated even though the front-lens of the substage condenser has been removed. In this case the following technique should be used:

The lamp should be placed about 40 cm. from the substage condenser iris.

The distance of the stand condenser from the lamp should equal its own focal length, i.e. 7 cm., to obtain a parallel beam of light.

A bi-convex lens with a focal length double that of the stand condenser, i.e. 14 cm., and having about the same diameter as the latter should be obtained. This lens, mounted on an independent stand, is to be employed as an *auxiliary stand condenser* and should be placed at a distance of 14 cm. (equal to its own focal length) from the substage condenser iris.

The auxiliary iris should be placed as close as possible to the stand condenser (not the *auxiliary* stand condenser) and its image focused in the plane of the object as in the previous examples. The whole field will now be found to be uniformly illuminated.

Another and perhaps easier way of illuminating the whole field of very low-power objectives is to employ one of the various special low-power substage condensers, such as Watson's Macro Illuminator.

Focusing the Camera

When all the adjustments previously described have been completed, the camera is placed in position and the microscope image is carefully focused on the ground glass focusing screen. This screen has a clear glass centre for high-power work when

the focusing must be carried out by means of a focusing-glass. The focusing-glasses commonly found on the market are far too strong; one magnifying 1 1/2 times, or at most twice, gives the best results. It should be noted that the image must be focused on the screen by means of the microscope drawtube, the correction collar (if the objective is provided with one) or a tube-length corrector to avoid disturbing the spherical correction of the objective after the correct adjustments for cover-glass thickness have been completed. The microscope fine adjustment mechanism should only be used for the final touches to the focusing.

Finding the Focal Length of a Lens

It may be useful for the microscopist to know how to ascertain the focal length of a stand condenser or a converging lens. The quickest method is the following:

An opaque screen, in which a small slit about 8 mm. (1/3 in.) by 3 mm. (1/8 in.) has been cut, is placed in front of a suitable light source such as an opal electric bulb.

The lens, whose focal length is to be measured, is brought opposite this slit and a plane mirror is placed behind the lens in question and in contact with it. The lens is held vertically and a reflected image of the slit is projected on the screen by the side of the slit itself. The lens is then moved towards or away from the screen until the reflected image and the slit are exactly the same size. The distance from the screen to the lens is measured and the figure thus obtained represents the focal length of the latter with a sufficient degree of accuracy for most purposes.

Some Final Hints on Photomicrography

The performance of achromatic objectives is considerably improved by employing approximately monochromatic light. The use of suitable colour filters has much to recommend it, especially with stained objects. Filters of the same colour as the object will give maximum detail, those of complementary colour maximum contrast. An intermediary tint usually gives the best all-round results.

The photographic plates used should be sensitive to the

colour of the filter selected. Fine-grained, orthochromatic, backed plates are the best.

Leave all apparatus in place with the illuminant turned on at least ten to twenty minutes, according to the light source, before making the exposure. Many plates are spoilt owing to variations in the focus due to expansion or contraction of the various parts of the microscope or camera caused by temperature changes.

Before completing the final focusing on the ground or clear glass screen, it is advisable to give the table near the microscope a few sharp taps to cause the various parts to 'settle' and allow the subsequent focusing to be more stable.

Vibrations are far less to be feared in photomicrography than sudden jerks or any other shock which may *disturb the objective's focus*; 90 per cent perhaps of the failures in photomicrography are due to this cause. For this reason, after having finished the exposure and removed the plate holder, it is advisable to replace the ground glass screen and check the focusing. If this is still sharp, the plate will probably be satisfactory; if the focus has changed, the negative will almost certainly be a bad one. Placing each leg of the work-table on a pile of unbound magazines helps to absorb floor vibrations before they can reach the apparatus.

All stray reflections must be carefully guarded against. No extraneous light should be allowed to fall on the objective mount or on the upper surface of the slide or stage.

3. METHODS FOR DETERMINING THE POSITION OF AN OBJECT

The position of any detail of interest in the slide can be permanently recorded for future reference by two principal methods:

1. By surrounding the object itself by a small circle or other mark inscribed on the cover-glass.
2. By taking the co-ordinates of the object by various means.

1. Determining the Position of an Object by Marking the Cover-glass

This method is the quickest and most certain, especially if the slide has to be sent to some other person for examination. The

object of interest can be permanently identified if a small circle is drawn round it on the cover-glass (or on the slide) by means of an accessory known as a Diamond Object Marker procurable from most optical firms.

If the microscopist does not possess this accessory, he can obtain similar results in the following manner:

The object to be recorded is brought to the centre of the field of a medium-power objective and the latter and the substage condenser focused in the usual way. The objective is then racked upwards and the slide is observed directly with the naked eye without removing it from the stage. A small bright disk of light, thrown by the substage condenser, is seen on the slide and this is made as small as possible by closing the condenser iris. With a fine pen dipped in Indian ink, three tiny spots are marked on the cover-glass in the form of an equilateral triangle, each spot tangential to the central bright disk. If the substage condenser has been correctly centred with the objective, it is evident that the object will be somewhere in the small area delimited by the three spots. This can be verified, if necessary, by re-examining the slide under the microscope. The disk of light can be made more visible if a thin piece of tissue paper is placed on the stage beneath the slide.

The three spots will last for quite a long time as Indian ink is fairly resistant to all reagents, including the action of cedar-wood oil. The slide can be marked in a more permanent manner by making a tiny scratch near each ink spot with a fine glazier's diamond.

2. Determining the Position of an Object by Taking its Co-ordinates by Means of the Mechanical Stage

This second method for determining the position of an object takes more time and requires a mechanical stage fitted with graduations and verniers. The position of the object is recorded from the readings of the horizontal and vertical motion verniers after the object has been brought into the centre of the field of a medium- or high-power objective.

The figures thus obtained are unfortunately useless for relocating the object on another microscope and are sometimes of

little help even on the same instrument if there is the slightest play in the component parts of the mechanical stage.

There are many other methods for recording the position of an object on a slide, but it is doubtful if there are any simpler or more effective than the first of the two referred to above.

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APPENDIX

DEFINITION OF TERMS USED IN MICROSCOPY

Aberration. Any deviation of the light rays emanating from one point of an object which prevents them meeting in a corresponding point of the image after passing through a lens.

Aberration, chromatic. Unequal bending of the light rays passing through a lens which causes rays of different wavelengths (i.e. of different colours) to meet at different foci. This results in a blurring of the image by the appearance of colour fringes around its outlines.

In an ordinary uncorrected biconvex lens, violet rays meet at a point nearer to the lens than red.

Aberration, spherical. Unequal bending of the light rays passing through the central and marginal zones of a lens and causing them to meet at different foci. This produces a general blurring of the image.

In an ordinary uncorrected biconvex lens, rays passing through the marginal meet at a point nearer to the lens than those passing through the central zones.

Achromatic. Free from chromatic aberration.

Angle of Aperture. The angle formed by the most oblique light rays which, diverging from the same point of an object, are capable of entering the front-lens of an objective.

Aplanatic. Free from spherical aberration.

Apochromatic Objective, Apochromate. A type of objective which is freer from chromatic aberration than the so-called 'achromatic' objective.

Back-lens, Condenser. The lens furthest from the object on the stage, i.e. nearest to the mirror.

Back-lens, Objective. The lens furthest from the object on the stage, i.e. nearest to the eyepiece.

Cover-glass. A piece of very thin transparent glass, usually square or circular, which is placed over the object on the stage.

Most optical firms correct their objectives for a cover-glass thickness of about 0·18 mm. (0·007 in.) or slightly less.

Diffraction. Changes in the direction and mode of vibration of a light ray after passing by the edge of an opaque body or through a narrow slit. The latter may be the interval between two details of an object possessing a periodical structure.

Field. The circular illuminated surface seen when looking through a microscope. The area of the object included in the field is smaller with high powers than with low.

Focal Length (*F.L.*). The distance between the centre of a lens (or the optical centre of a system of lenses) and the point where a parallel beam of light is brought to a focus. The focal length is shorter with high-power objectives than with low.

Focal length should not be confused with *working distance* (see definition). An objective of 4 mm. F.L. has, at maximum, a working distance of only 1 mm.

Front-lens, Condenser. The lens nearest to the object on the stage.

Front-lens, Objective. The lens nearest to the object on the stage.

Magnification. The power of a lens to increase the apparent size of an object.

Magnification, combined. The magnification obtained by multiplying the initial magnification of an objective by the initial magnification of the eyepiece with which it is used.

In practice this term has really come to mean the magnification obtained by multiplying the *partial* magnification of an objective by the initial magnification of the eyepiece.

Magnification, empty. An increase in the apparent size of an object without the appearance of any fresh detail; i.e. magnification without resolution. See page 37.

Magnification, initial. The enlargement of the real image projected by a lens or objective at a distance of 250 mm. (10 in.).

Magnification, partial (*of an Objective*). The enlargement of the real image projected by the objective in question at the

distance of the lower focal plane of the eyepiece with which it is used. See page 27.

Numerical Aperture (*N.A.*). See page 28.

Numerical Aperture, effective. See page 42.

Slide, Slip, Object-slip. A thin transparent piece of glass, 3 × 1 in., on which the object is mounted for microscopical examination.

Most opticians correct their condensers to work through a slide 1—1·5 mm. thick.

Over-correction, chromatic. A condition in which a system of lenses brings the rays towards the red end of the spectrum to a common focus, but not those towards the violet end.

Over-correction, spherical. A condition in which a system of lenses brings the rays passing through its marginal to a more distant focus than those passing through its central zones.

Optical Index. See page 38.

Penetration. The power of a lens or objective to see more than one plane of an object in sharp focus at the same time. Penetration diminishes as the numerical aperture increases.

Reflection. Change in the direction of a light ray after meeting a non-transparent and non-absorbing obstacle. The angle of reflection is equal to the angle of incidence.

Refraction. The bending of a light ray when passing through a lens, or from one medium to another of greater or lesser density.

Refractive Index (*R.I.*). The ratio of the sine of the angle made by the incident light ray (with a perpendicular to the refracting surface) to the sine of the angle made by the refracted ray (with a similar perpendicular). Air is taken as unity when calculating the refractive index of any transparent substance.

Resolution. The power of a lens to reveal the most delicate details of an object. The resolution of an objective increases with its numerical aperture.

Secondary Spectrum. The residual colour fringes shown around the outlines of an object examined with an ordinary achromatic objective.

With a good objective these only become obtrusive under oblique or dark-ground illumination.

Tertiary Spectrum. The residual colour fringes shown by an apochromatic objective. These can only be detected under the most stringent conditions and with the most delicate objects.

Tube-length, mechanical. The distance between the upper end of the drawtube, where the rim of the eyepiece rests, and the lower end of the body-tube into which the objective is screwed.

Most opticians correct their objectives for a mechanical tube-length of 160—170 mm. This includes the length of a revolving nosepiece or any other pattern of objective-changer that may be used.

Tube-length, optical. The distance between the upper focal plane of the objective and the lower focal plane of the eyepiece.

Under-correction, chromatic or spherical. The inverse of chromatic and spherical over-correction.

Working Distance. The distance between the front-lens of an objective and the object on which it is sharply focused. The working distance diminishes as the numerical aperture increases.

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